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CORRECTIONS

Volume 38, 1960

Page 2159, line 8 up. For "0.998 *N*", substitute "0.0998 *N*".

Volume 39, 1961

Page 599, line 1. After ". . . are positive", insert "and of bimolecular acid-catalyzed reactions are negative".

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VOLUME 39 OCTOBER 1961 NUMBER 10

SURFACE EXCHANGE REACTIONS OF SILVER AND ITS IONS[1]

J. E. SANDOR[2]

ABSTRACT

The exchange reactions between silver and its ions in solution have been investigated by the use of radioactive tracers. Silver of highest purity was cleaned carefully and immersed into a radioactive silver nitrate solution. The course of the early absorption process and subsequent diffusion of the ions into the interior of the metal was followed in detail, and the diffusion coefficient at room temperature was calculated. The penetration depth was determined by controlled electropolishing of the metal, and the reverse movement of active ions from the metal into inactive solutions was also observed.

INTRODUCTION

The exchange reaction between metallic silver and its ions in solution has been investigated by several authors. This metal is of particular interest because it is available in high purity, is chemically inert, and has a convenient radioactive isotope with a half-life of 270 days.

Rollin (1) found that metallic silver, shaken with a silver nitrate solution containing radioactive silver, acquired an activity corresponding to more than 100 apparent atomic layers of silver. Baerg and Winkler (2) found that "abraded" and etched foil exchanged to different depths. They measured the "true" area by the Bowden–Rideal method (3) and tried to correlate surface area and exchange, but concluded that "the metal surfaces were poorly reproducible in respect to both exchange and area". Gerischer and Vielstich (4) observed that after a rapid initial exchange, which depended on the surface treatment, a second, slower increase in observed activity took place, and they attributed this increase to solid-state diffusion.

Tingley, Henderson, and Coffin (5) concluded from their work that generally there are two mechanisms acting in silver exchange reactions, one "kinetic" and the other a "true exchange"; which one of these was acting depended on the state of the surface.

King and McKinney (6) recently also found evidence that solid-state diffusion occurs in silver, accompanying the exchange reaction. A theoretical study of the heterogeneous reaction was done by Zimens (7). He devised equations relating the progress of the solid–liquid exchange with time, assuming that one of the following processes is the rate-determining one: (1) diffusion across the interface, (2) chemical reaction at the phase boundary, and (3) diffusion of the exchanging atoms into the interior of the solid. The rate laws for these processes are formally similar and are usually of an exponential nature.

It was the purpose of the present experiments to study the exchange between silver and its ions, following Zimens' model.

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Contribution from the Mineral Sciences Division, Mines Branch, Department of Mines and Technical Surveys, Ottawa, Canada.

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The main questions to be solved were: (1) the probable mechanism for a fast exchange to a depth of more than 100 atom layers, (2) the cause of the slow increase in observed activity after the initial exchange, and (3) a decision as to which of the three processes in Zimens' model were the rate-controlling ones. Gerischer and Vielstich (4) assumed that self-diffusion is the controlling step in the exchange; King and McKinney found some penetration of the radioisotope after the exchange. To confirm or deny these authors' findings and assumptions, an electropolishing technique was developed to measure the penetration of the radioisotope inside the solid.

Three major practical factors were considered essential to the success of the experiments: (1) use of high purity materials, (2) avoidance of oxidation and corrosion, and (3) careful preparation of the surfaces.

It should be stressed that small variations in technique can affect markedly the results of all such exchange experiments.

EXPERIMENTAL DETAILS

The radioisotope utilized for all the experiments was silver-110, obtained from Atomic Energy of Canada Ltd. It has a half-life of 270 days, and a complex beta- and gamma-ray spectrum with a maximum beta energy of 2.8 Mev and a maximum gamma-ray energy of 1.516 Mev.

All the activity measurements were done with a well-type NaI(Tl) scintillation counter. A statistical analysis of the counting assembly was done using a known source of Ag-110; the test indicated a satisfactory operation of the unit.

A 10^{-2} M silver nitrate solution was prepared with a specific activity of 6.5 μc/cc and a pH of 6.6. Metal foils were prepared from Johnson-Matthey 99.999% silver. Rods of 1.9-cm diameter and 2.5-cm length were alternately cold-rolled to 50% reduction and annealed 15 minutes at 500° C, to a final thickness of 0.0025 cm.

From these foils, samples of 1.5 cm^2 area were cut in such a way as to leave a short tip on one of the sides to use as a holder during the handling of the samples. (Silver-plated tweezers were used during all the experiments.)

These foils were cleaned in boiling ethanol for 1 hour, electropolished in a cyanide bath for 10 minutes, annealed for 20 minutes at 400° C, and finally electropolished again for 10 minutes. Samples prepared in this way showed no surface deformation when X-ray diffraction pictures were taken. These samples also stayed bright and untarnished when stored for considerable periods.

It was found that high annealing temperatures or long annealing periods produced some thermal etching, and that the pits were noticeably broadened during electropolishing.

EXPERIMENTS ON EXCHANGE AS A FUNCTION OF TIME

To find out if there was a continuous increase in activity after long periods of immersion,* 12 samples were left in a 100-cc Petri dish containing 50 ml of radioactive solution. The Petri dish was kept, for the duration of the experiments, in a well-shielded lead castle.

To simplify the handling of the specimens, they were attached by their tips to a ring of Lucite with a drop of paraffin. In this way some samples were immersed for as long as 37 days. This arrangement was adopted to avoid concentration changes in the solution

*By "long" is understood more than 1 day.

by evaporation, and at the same time to make it possible to subject at least 12 samples simultaneously to exchange processes.

The solution was not stirred; it was assumed that even without agitation the diffusion through the diffusion layer in the liquid was much faster than the solid-state diffusion in the metal and so this factor would not influence the final results. (The ratio $D_l/D_s = 10^{12}$.)

The samples were taken out at regular intervals, rinsed carefully with cold water and alcohol, dried, and counted in the scintillation counter. The first counting was done after 9 days of immersion. No counting was done at the beginning of the exchange, because previous experiments had established a very rapid increase in activity during the first few minutes of the exchange. In the work described here the emphasis was on the longer-term exchange, particularly to show whether there is a continuous increase in activity in the samples, and whether this slow increase is due to solid-state diffusion.

A small correction had to be made to take into account the decay of the radioisotope.

EXPERIMENTAL RESULTS

Activity Increase as a Function of Time

The results of the long-term immersion tests are plotted in Fig. 1 in terms of apparent

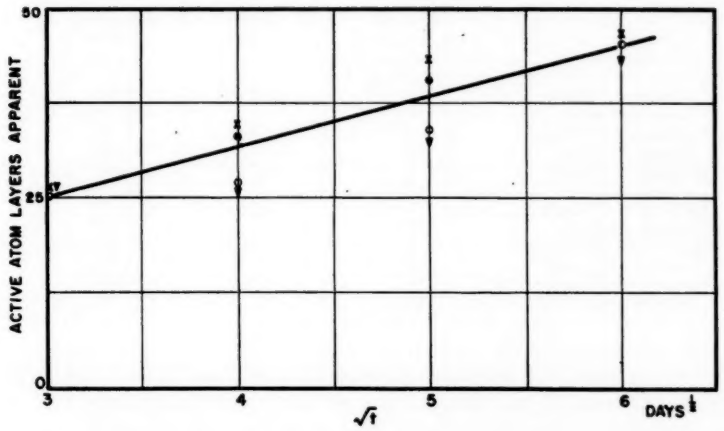

FIG. 1. Exchange as a function of time of immersion: ×, ●, ▼, ○ represent different samples.

layers of exchanged atoms as a function of time of immersion. The relation between adsorbed activity and equivalent atom layers had been established in an auxiliary electropolishing experiment. The first count was obtained after 9 days of immersion, and subsequent ones at intervals of 3 or 4 days.

The points correspond to different samples, but it seemed better to plot the average curve of the different samples than to draw a curve for each sample. There is some scatter in the points, but this was not the case when each sample was counted individually.

From this curve it can be seen that the activity increases approximately as a function of the square root of time, as expected if a diffusion process is the rate-determining process.

In the case of three samples the activity pickup was much higher than for the others. The reason for this divergence is not clear, but it may have been caused by some disturbance of the adsorption equilibrium, or by a deformation of the surfaces during the handling, which interfered with the reaction exchange – solid-state diffusion. This curve does not fit the equation $A_t = f(\sqrt{t})$.

Measurement of Penetration Depths

To find out if there was a measurable penetration of the radioactive isotope, and to calculate a self-diffusion coefficient for silver, the "exchanged" specimens were electropolished under reproducible conditions. It was ascertained that the same period of electropolishing removed the same weight of silver each time. In addition to this, before electropolishing, the samples were immersed in the electrolyte for short periods of time—ranging from 1 second to 1 minute—without applying electrical current, to see which portion of the activity was "re-exchangeable". The remaining activity in the samples was then counted.

In Table I are summarized the results of the immersion of the samples in the electrolyte

TABLE I

Exchanged samples immersed in the electropolishing solution without applying current

Sample No.	History	Time of immersion in electrolyte, sec	Initial activity, c.p.m.	Remaining activity, c.p.m.	Loss of activity, %
1	Exchanged 34 days, stored 60 days	1	14,000	8,900	36
		60		7,000	50
2	Exchanged 34 days, stored 30 days	1	12,000	7,700	36
		60		6,100	49
4	Exchanged 37 days, stored 60 days	1	25,000	18,000	28
		60		14,300	43
6	Exchanged 31 days, stored 60 days	1	13,600	7,700	43
		60		6,800	50
8	Exchanged 25 days, immediately after	1	25,000	19,200	23
		60		14,600	42
9	Exchanged 25 days, immediately after	1	24,600	18,000	27
		60		10,600	57

without applying current. It is evident that more than 40% of the activity is "exchangeable", and that between 20 and 45% of the activity is lost after the first second of immersion; in addition to this, the samples that picked up higher activity have a higher fraction of "exchangeable" atoms.

The results of the experiments done by progressively electropolishing the exchanged samples show that there is a measurable penetration of the radioactive tracer.

In Fig. 2*a* are plotted the penetration curves of some of the samples listed in Table I. The logarithm of the activity in c.p.m. is plotted as a function of the time of electropolishing. It was established by previous experiments that, under the operating conditions used, in the exchanged samples 1 minute of electropolishing was equivalent to removing an amount of silver equivalent to a thickness of 1μ.

It was observed that there was a large decrease of activity after removing the first 0.13 to 0.25 μ, and then a much smaller decrease in activity when a subsequent comparable thickness was removed.

It can be seen in Fig. 2*b* that the penetration curves are clearly the combination of two

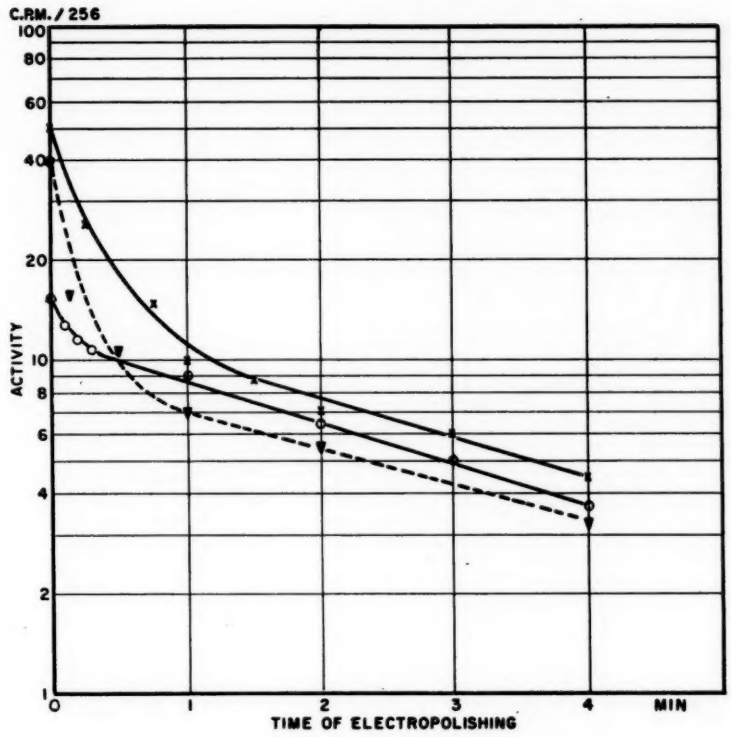

FIG. 2*a*. Penetration curves for fresh samples: ○, sample No. 2; ×, sample No. 8; ▼, sample No. 9.

independent exponential functions representing two processes proceeding at different rates.

It was observed that long storage of the foils after the exchange process affected the slope of the second part of the curves, and it made some difference in their initial slope. This effect is shown in Fig. 3. Longer storage times tended to flatten this first part.

CALCULATION OF DIFFUSION COEFFICIENT

From the data of the penetration curves the self-diffusion coefficient of silver at room temperature was calculated.

Fisher (8) made a mathematical analysis of the relation between grain boundary diffusion D_b and volume diffusion D_v by assuming that grain boundary diffusion is analogous to the diffusion of heat along a thin copper foil embedded in cork. He arrived at an expression

$$D_b = 2D_v^{1/2}(\log e)^2 \Big/ \delta\left(\frac{d\log a_x}{dx}\right)^2 (\pi t)^{1/2},$$

where e is the logarithm base and δ the grain boundary thickness. The same author

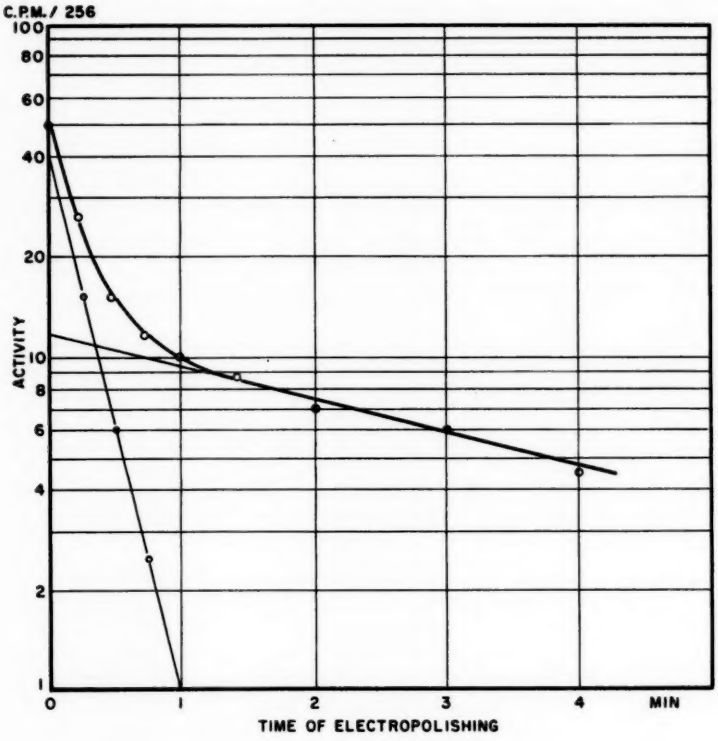

FIG. 2*b*. Penetration curves: analysis of typical curve.

assumed for δ a value of 5×10^{-8} cm that has since been criticized (9) because it is very hard to establish a uniform thickness for the grain boundaries of metals, but it can be used as an approximation. D_v can be calculated from the formula of Hoffman and Turnbull (10):

$$D_v = 0.895\exp(-49{,}500/RT)\ \text{cm}^2/\text{sec};$$

the value at 25° C is $D_v = 9.8\times10^{-38}$ cm²/sec. Inserting this value into the equation for D_b gives

$$D_b = 6\times10^{-11} \Big/ \left(\frac{d\log a_x}{dx}\right)^2 t^{1/2}$$

$$= 1.37\times10^{-20}\ \text{cm}^2/\text{sec},$$

where a_x = activity, x = penetration, and t = time of exchange. The slope of the straight part of the curves of Fig. 2*a* and a value of t = 34 days were used for the calculation.

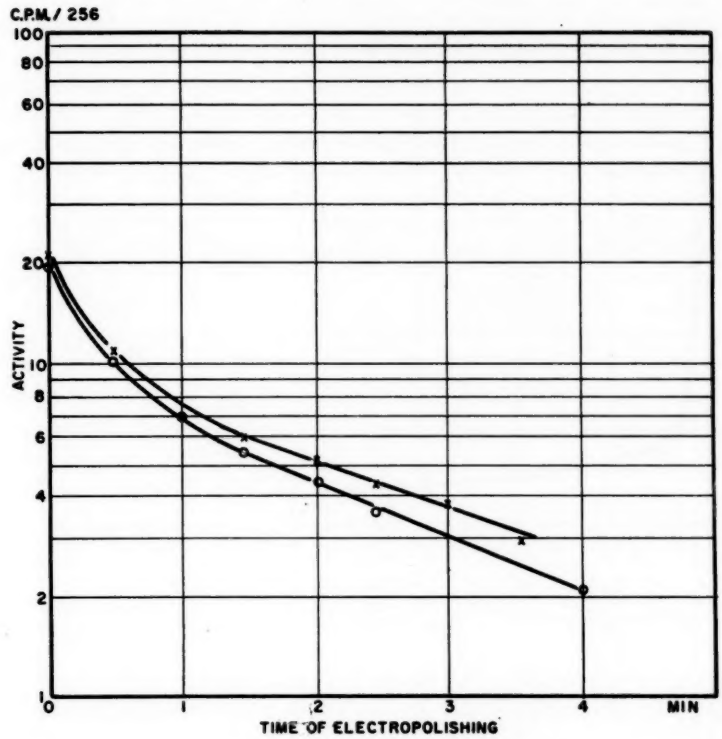

FIG. 3. Penetration curves for aged samples: ×, sample No. 11; ○, sample No. 12.

DISCUSSION AND CONCLUSIONS

The results of this work confirm the conclusions from previous work that self-diffusion of silver is the rate-controlling step in the exchange reaction, and show clearly that there is fast diffusion in the uppermost atomic layers (approximately 300 layers). In the absence of corrosion, oxidation, or local cell action, in the case of silver a rapid exchange takes place between the surface atoms of the metal and the ions in the liquid on immersing the electropolished foils in the exchange solution. At the same time, some local cell action due to the presence of different crystal faces may take place, but in a polycrystalline material the electromotive force resulting from this difference must be negligible.

To measure the extent of reversibility of this exchange reaction, the reverse experiment was carried out. It was found that close to 50% of the activity of the samples was lost after a few seconds of immersion in an inactive silver nitrate solution. This would indicate that the adsorbed (chemisorbed and ionic double-layer) atoms re-exchange readily with the solution; the results are plotted in Fig. 4.

The results of the long-term experiments on the increase of activity with time gave a clear indication that a diffusion process was involved. The penetration curves confirmed this supposition. It was found that fast exchange at the surface is followed by diffusion of the atoms inside the solid.

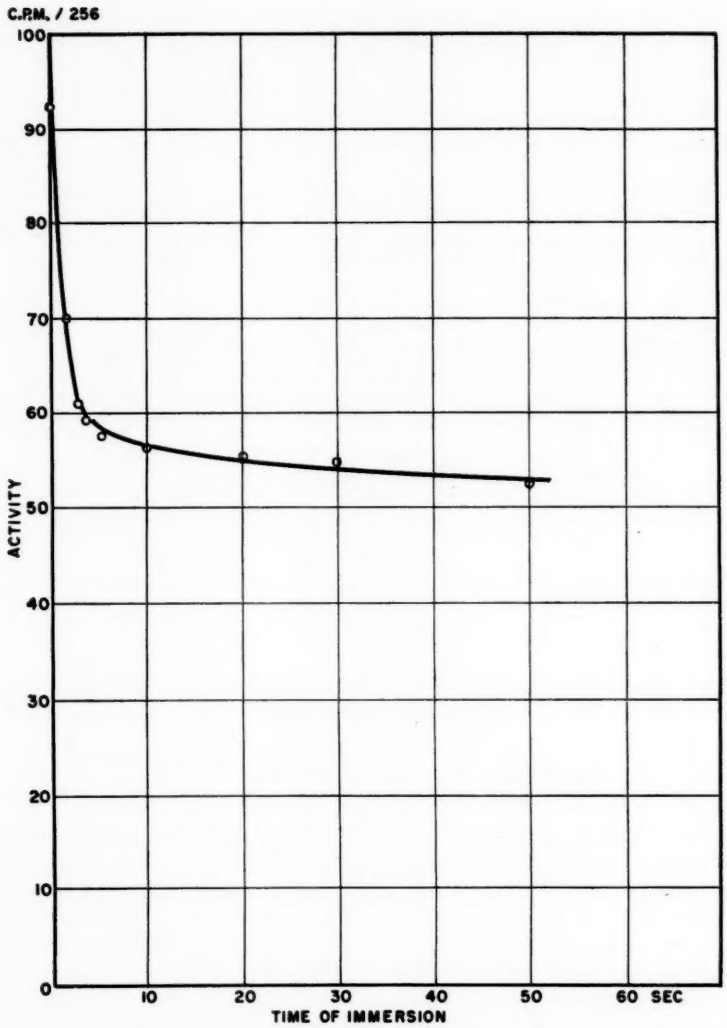

FIG. 4. Reverse exchange, active metal immersed in inactive solution.

It follows that two different mechanisms are acting, a fast diffusion process down to a depth of 300 atomic layers, followed by a much slower one at greater depth.

The diffusion coefficient found in the present study for this second process is $D_b = 1.37 \times 10^{-20}$ cm²/sec. This compares with a value of $D_b = 6.14 \times 10^{-22}$ cm²/sec, using Hoffman and Turnbull's formula and calculating for 25° C of temperature.

The difference of two orders of magnitude can be explained either as a result of extrapolation, because the work of Hoffman and Turnbull was done at high temperatures, or

because there is a difference in the activation energy, indicating that another mechanism is acting at low temperatures.

Other authors found a difference of 4 or 5 orders of magnitude; this can be attributed to less-pure samples, or to less-well-polished surfaces.

It is an accepted theory that self-diffusion in face-centered cubic metals takes place by a lattice-vacancy mechanism. It has already been shown that in diffusion curves for polycrystals unusually high activity was noted at small penetration depths (11). The same result is found in the present study, so it is probable that there is a much more disordered and loose structure of atoms close to the surface exposed to the solution.

This would explain the high rate of adsorption at the beginning of the exchange reaction. It also accounts for the fact that in cold-rolled samples there is a faster diffusion than in annealed specimens, and for the observation that pretreating the surfaces in inactive silver before the exchange experiments tends to decrease the exchange rate.

The whole process can be pictured as in Fig. 5, i.e., similar to the interdiffusion of two solids.

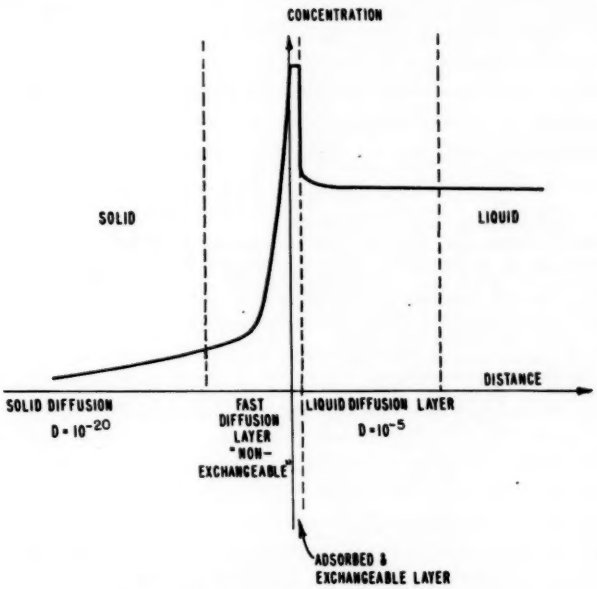

FIG. 5. Diagram of exchange layers.

The smallest diffusion coefficient is in the solid phase and it is the rate-determining factor in the whole exchange process under near-equilibrium conditions at the surface.

The atoms move from a solid lattice structure through a "fast diffusion" layer, through a phase boundary, through the liquid diffusion layer, and finally to the bulk of the liquid.

Summary

(1) A continuous increase in activity was found by immersing electropolished and annealed silver foils in a silver nitrate solution containing radioactive silver, for periods of 9 to 37 days. The adsorption process was a square root function of time.

(2) Close to 50% of the activity was lost by reverse exchange when the exchanged samples were immersed in inactive silver nitrate solution; the remainder was removed from the contact area by diffusion into the interior of the metal.

(3) The measurement of the penetration of the radioactive isotope after exchange was carried out using an electropolishing technique and showed an exponential distribution with depth.

It is concluded that a fast diffusion occurs close to the surface, followed by a slower one inside the metal. The calculated diffusion coefficient in the bulk of the solid does not differ much from the one calculated from Hoffman and Turnbull's results.

(4) A model of the whole exchange process is proposed, assuming that self-diffusion in the solid is the rate-determining process.

ACKNOWLEDGMENTS

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CLAM POISON

II. PURIFICATION OF CLAM POISON RESIDUES OF LOW TOXICITY BY A HEAVY-PAPER TECHNIQUE[1]

R. A. B. BANNARD AND A. A. CASSELMAN

ABSTRACT

The usefulness of Whatman seed-test and No. 17 papers has been examined for purification and recovery on a preparative scale of the toxin present in residues of low toxicity from the Schantz alumina chromatographic method for purification of clam poison. A method was developed to minimize introduction of impurities from the papers into fractions recovered after chromatography when *t*-butanol:acetic acid:water (2:1:1) was used as developer. Seed-test paper gave better separation of the toxin from other components of the residues than did No. 17 paper. Residues of bio-assay as low as 300 MU/mg were found to be capable of enrichment by the method. On a scale of 500 mg per 18×22-in. sheet of pretreated seed-test paper, using residues of toxicity 1400 MU/mg, a 67% recovery of toxin enriched fourfold to 5800 MU/mg was achieved in four passes. Increased loading of the paper led to a decrease in both enrichment per pass and over-all recovery of toxin. The paper chromatographic properties, toxicity, and infrared spectrum of the recovered poison agree with those reported by Schantz and co-workers for pure clam poison dihydrochloride, but the specific rotation is lower by 25%. Four possible alternative explanations for the observed anomalous rotation are discussed.

INTRODUCTION

In the method devised by Schantz and co-workers (1) for purification of clam poison, the final step involves chromatography of clam poison dihydrochloride of 55–70% purity on acid-washed alumina, employing absolute and aqueous ethanol as eluants. In this step, 25–45% of the toxin applied is recovered in pure form and an over-all recovery of 70–90% is achieved, but some of the toxin remains on the column and must be removed by elution with water. Some of the fractions resulting from the process proved capable of further enrichment by recycling in the same manner, but it was not found profitable to attempt enrichment of samples of purity less than 30–50% in this way (2). In recent years, considerable quantities of clam poison residues of low toxicity from this process (hereinafter referred to as clam poison tailings) have accumulated, and it has been estimated (3) that as much as 25 g of toxin could be made available for study if a suitable method of purification could be devised. Several attempts were made to enrich clam poison tailings by chromatography on paper, cellulose columns, cation exchange resins, and acid-washed alumina, but these proved fruitless (4). The successful application of paper chromatography to this problem on a semimicro scale, which we recently reported (5), led us to attempt extension of the method to a preparative scale by the use of heavy paper. The present communication describes a method whereby clam poison tailings of toxicity 1400 MU/mg can be enriched to toxicity 5800 MU/mg in 67% yield on a scale of 500 mg.

RESULTS AND DISCUSSION

The purification of clam poison dihydrochloride and clam poison tailings by chromatography on a semimicro scale on Whatman No. 1 paper using *t*-butanol:acetic acid:water (2:1:1) as developer has recently been described (5). In this work extensive pretreatment of the paper was necessary to minimize the introduction of impurities into the recovered toxin. Brownell, Hamilton, and Casselman (6) previously established the usefulness of

[1]*Manuscript received May 18, 1961.*
Contribution from Defence Research Chemical Laboratories, Ottawa, Canada.
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chromatography on Whatman seed-test paper for the purification of amino acids and pyrophosphate esters on a preparative scale and found extensive pretreatment of the paper unnecessary since no appreciable quantities of impurities were introduced into the recovered substances by the neutral solvent systems which they employed. We were committed to use of the acidic solvent system mentioned above because of its ability to separate the components present in clam poison tailings and it seemed to us that the effectiveness of the heavy-paper technique would depend largely on the quantities of impurities introduced into the various fractions during chromatography. The other factor upon which the usefulness of the method would primarily depend is the degree of resolution of components attainable relative to the loading of the paper.

Attention was first directed to the question of impurities introduced during chromatography and the steps required to minimize them. Untreated Whatman No. 17 and seed-test papers were developed in *t*-butanol:acetic acid:water (2:1:1) by the descending method and air-dried; rectangular strips corresponding to preselected R_F ranges were removed and eluted with water; and the quantities of extractable impurities were determined by weighing, after lyophilization. Papers were then pretreated by the chromatographic washing procedure which had proved to be most effective for the removal of impurities from Whatman No. 1 paper (5) and the quantities of residues recovered by aqueous elution were determined as described above. Finally, the residues thus obtained were taken up in water and the quantities of impurities remaining after (*a*) filtration through a fritted glass filter and (*b*) elution through Amberlite IRA-400 (Cl) resin were determined.

It is evident from Table I that, as anticipated, unwashed seed-test paper yields much

TABLE I

Impurity recovered from Whatman seed-test and No. 17 papers

R_F range of band	Weight recovered by simple elution (mg/in²)		Weight after filtration (mg/in²)		Weight after resin treatment (mg/in²)	
	Unwashed	Pretreated	Unwashed	Pretreated	Unwashed	Pretreated
Whatman seed-test						
0–10	0.317	0.054	0.285	0.038	0.223	0.024
14–25	0.563	0.061	0.535	0.040	0.438	0.026
29–39	1.151	0.061	1.097	0.035	0.984	0.025
40–51	2.010	0.061	1.946	0.043	1.791	0.026
55–65	2.960	0.058	2.899	0.040	2.760	0.027
79–90	3.363	0.076	3.256	0.058	3.085	0.035
Whatman No. 17						
0–10	0.086	0.057	0.079	0.037	0.041	0.028
14–25	0.092	0.038	0.072	0.031	0.047	0.022
29–39	0.143	0.041	0.132	0.029	0.084	0.016
40–51	0.175	0.050	0.162	0.026	0.089	0.017
55–65	0.645	0.059	0.605	0.028	0.234	0.017
79–90	0.736	0.058	0.734	0.036	0.353	0.021

larger quantities of impurities than does No. 17 paper. The quantity of impurity in both unwashed papers increases rapidly with increasing R_F, in agreement with results obtained earlier with unwashed Whatman No. 1 paper. Pretreatment reduces the quantities of impurities extracted from both papers to the same order of magnitude and leads to greater uniformity in the weight of impurity as the R_F is increased. In the R_F range 0.14–0.39, which is the region in which clam poison was expected to be located in actual

chromatograms, pretreated No. 17 and seed-test paper gave quantities of impurities which were respectively three and four times as large as those found for Whatman No. 1 paper by simple elution. Portions of these impurities can be eliminated from the reconstituted aqueous eluates by filtration through a fritted disk. The relative improvement thereby produced is in the following order: pretreated No. 17 ≈ pretreated seed-test > unwashed No. 17 ≈ unwashed seed-test. These results indicate that pretreatment produces a relatively larger quantity of insoluble fibrous debris than was present in the unwashed paper, but effectively removes the majority of the impurity initially present, which was mainly soluble rather than fibrous. More impurities can be eliminated from the reconstituted aqueous eluates from the filtration treatment by passing the solution through Amberlite IRA-400 (Cl) resin. The relative improvement was approximately identical for all but unwashed seed-test paper. These results suggest that the type of anionic impurity which is being removed from all but unwashed seed-test paper is uniform and this impurity could well be acetate ion which had been introduced by the pretreatment. The type of anionic impurity present in unwashed seed-test paper would be expected to be less uniform since the paper received no pretreatment.

By combined pretreatment, filtration, and elution through resin, the impurities in the R_F range 0.14–0.39 for No. 17 and seed-test papers were reduced respectively to one and one-half and twice the quantities obtained from pretreated Whatman No. 1 paper. Attempts were made to reduce the quantities of impurities still further by the use of millipore filters but no improvement resulted. Huffman and co-workers (7) found during alternate successive aqueous extraction and drying of Whatman No. 1 paper that no further reduction in the water-soluble carbohydrate impurities recovered occurred after the third extraction. This phenomenon was considered (7) to be related to the deterioration of cellulose caused by alternate wetting and drying of the paper and by the action of ozone at high moisture levels. Since the pretreatment method which was used for the purification of Whatman No. 1 paper (5) and the papers in the present investigation involved air-drying prior to water-washing after treatment with each irrigant, it seemed worth while to investigate the effect of eliminating the drying steps by using a continuous washing procedure. The quantities of residues found by this method after subsequent filtration and resin treatment were approximately one-third larger than given by the intermittent washing procedure and therefore no improvement resulted. Continuous washing did have the advantage, however, of reducing the time required for washing the papers from 39 to 30 days.

As a result of the purification of the heavy papers it was estimated that if a toxin fraction as large as 100 mg could be confined to a rectangular band 1×18 in., only 0.3 to 0.4% of impurity would be introduced by the paper, and this level of impurity was considered to be quite acceptable for our purposes.

Preliminary chromatograms were performed on Whatman No. 1, No. 17, and seed-test papers using clam poison tailings of toxicity 1400 MU/mg (25%) and $[\alpha]_D^{25}$ +26.3°. The results given in Table II indicate the presence of six components, only three of which are Weber-positive (8). The atypical Sakaguchi (9) and ninhydrin reactions shown by component 2 were observed earlier (5) to be characteristic of clam poison and it was concluded that this was the desired material. The components were sufficiently well separated in these chromatograms to suggest that successful separations could be achieved at considerably heavier loading.

A sample of clam poison tailings (100 mg, toxicity 1400 MU/mg, $[\alpha]_D^{25}$ +26.3°) was applied to a $4\frac{3}{4}$×22-in. sheet of specially pretreated Whatman seed-test paper and

TABLE II

Qualitative paper chromatography of clam poison tailings

Component number	R_F on Whatman			Color with chromogenic agent			
	No. 1	No. 17	Seed-test	Weber	Sakaguchi	Ninhydrin	Ultraviolet
1	0.26	0.28	0.19	Pink	Red	Purple	Blue (f)
2	0.32	0.34	0.23	Pink	Yellowish green	Yellow	Blue (s)
3	0.38	0.38	0.29	Pink	Red	Purple	Blue (f)
4	0.40	0.44	0.34				Blue (f)
5	0.53	0.58	0.40				Blue (f)
6	0.64	0.74	0.53				Blue (f)

NOTE: (s) = strong, (f) = faint.

developed with *t*-butanol:acetic acid:water (2:1:1). The components were located on the air-dried chromatogram by their fluorescence under ultraviolet irradiation and were recovered by elution with water, and the eluates lyophilized, weighed, and bio-assayed as described in the experimental section. In this manner, fractions 1–6, given in Table III, were obtained. All the toxic material was confined to fractions 1, 2, and 3 and although

TABLE III

Quantitative paper chromatography of clam poison tailings*

Pass No.	Fraction No.	R_F	Corrected weight (mg)	Toxicity (MU/mg)	Total MU		% recovery
					Interim	Final	
1	1	0.18	17.40	800	13,900		
	2	0.23	23.30	4,400	103,000		
	3	0.27	35.37	800	28,400		
	4	0.34	24.00	Nil			
	5	0.42	1.21	Nil			
	6	0.50	0.69	Nil			
2	7	0.19	12.55	400		5,020	3.6
	8	0.24	12.20	2,900	35,400		
	9	0.30	28.10	100		2,810	2.0
3	10	0.19	3.71	1,000		3,710	2.7
	11	0.25	3.44	4,800	16,500		
	12	0.31	5.29	400		2,120	1.5
4	13	0.19	7.32	1,100		8,050	5.8
	14	0.25	16.10	5,800		93,400	66.9
	15	0.31	3.49	200		700	0.5
						116,000	83.0

*100 mg tailings (1400 MU/mg) applied (total MU = 140,000).

the percentage recovery on both a weight (102%) and toxicity (104%) basis was slightly greater than theoretical, the values found are within the experimental error of the method. The procedure is clearly capable of effecting a significant enrichment of clam poison present in the tailings since, judging from its toxicity, fraction 2 has been enriched threefold and contains 73% of the toxin originally applied to the paper. Fractions 1 and 3, on the other hand, are less pure than the original tailings and provided suitable material for examination of the effectiveness of the procedure for enrichment of tailings containing toxin approximately half as pure as that in the original sample. Fractions 1 and 3, after repurification as described above, gave fractions 7, 8, and 9. As before, the majority of

the toxicity (80%) was confined to the fraction of R_F 0.24, which had been enriched 3.5-fold to a toxicity of 2900 MU/mg. Although the degree of enrichment was slightly greater than that obtained in the first pass, the purity of fraction 8 is considerably less than that of fraction 2 because of the differential in purity of the two samples employed. Fractions 7 and 9 contain only 8000 MU of poison, which is little more than 1 mg of pure toxin (assuming the toxicity of pure clam poison to be 5500 MU/mg), and these fractions were discarded. In a subsequent experiment, however, in which 166 mg of tailings of bio-assay 300 MU/mg was purified by the same method, a 70% recovery of poison enriched approximately sevenfold to 2000 MU/mg was obtained, indicating that the procedure is effective even for concentration of tailings which contain as little as 5% of the poison. Fraction 8 was repurified in the same manner, yielding fractions 10, 11, and 12. In this pass, only an approximately twofold enrichment resulted and both the over-all recovery (64%) and percentage recovery in the most toxic band (47%) decreased sharply. By means of passes 2 and 3, however, the over-all recovery of poison of toxicity 4400 MU/mg or greater was increased to 85% from 73%. Fractions 2 and 11 were combined for a final purification to determine whether poison of toxicity identical with that reported by Schantz *et al.* (1) for pure clam poison dihydrochloride could be obtained by the method. The results given for fractions 14, 15, and 16 demonstrate that this objective was achieved.

In four treatments on seed-test paper it was thus possible to effect a fourfold enrichment of the toxin in 67% yield and to recover a further 17% of the poison in a less pure condition. Translating these results from $4\frac{3}{4}\times22$-in. strips to 18×22-in. sheets indicated that the method should be capable of treating 500 mg of tailings per sheet. In a subsequent experiment 500 mg of tailings was purified in the same manner as described above and produced identical results.

In another experiment the loading was increased to 200 mg per $4\frac{3}{4}\times22$-in. strip (i.e. 1.0 g per 18×22-in. sheet). In the first pass, 87% of the toxicity was recovered in the band of R_F 0.24, but the enrichment achieved was only 1.9-fold from 1400 MU/mg to 2600 MU/mg. After the fourth pass, 56% of the toxin applied in the first step was recovered, enriched 3.6-fold to 5000 MU/mg, and an additional 25% of the poison was recovered at lower enrichment levels. Thus, the lighter loading (500 mg per 18×22-in. sheet) is capable of producing more efficient enrichment than the heavier loading.

The usefulness of pretreated Whatman No. 17 paper in this method of purification was also examined, but was found less satisfactory than seed-test paper on the basis of both degree of enrichment and over-all recovery of toxin.

Previously, it was shown that clam poison dihydrochloride which had been purified by the semimicro paper chromatographic method (5) possesses a toxicity and rotation within the range of values reported by Schantz and co-workers (1, 10, 13) (5500 ± 500 MU/mg, $[\alpha]_D^{25}+130°\pm5°$) for the pure material. Also the infrared spectrum (KBr pellet) of toxin purified by the paper chromatographic method (5) was indistinguishable from that found by Schantz and co-workers. Samples of clam poison which bio-assayed at 5000–5800 MU/mg after purification by the heavy-paper technique described above were also found to exhibit an infrared spectrum indistinguishable from that previously reported for clam poison dihydrochloride (5) and we were therefore surprised to find that the specific rotations of these samples fell within the range 94–101°, which is well below the limit accepted for pure clam poison. Rechromatography of these samples on pretreated Whatman No. 1 paper gave only one Weber-positive spot, R_F 0.32 (cf. Table II). When ninhydrin was used as the chromogenic agent, however, very faint purple spots, R_F 0.26 and 0.38, were also revealed and very faint red spots with the same R_F's

were observed with Sakaguchi reagent. It thus appears that traces of components 1 and 3 (Table II) still remain in toxin (component 2) purified by the heavy-paper technique even when the bio-assay exceeds 5000 MU/mg. The intensities of the spots revealed by ninhydrin and Sakaguchi reagents were very weak, however, and it was considered unlikely that sufficient of these materials were present to markedly depress the rotation of the samples unless these components possess a high negative rotation. That such is not the case was determined by examination of side bands from the purification which are rich in the ninhydrin-positive components 1 or 3 and also contain a small amount of the Weber-positive toxin (component 2). The first fraction examined had toxicity 600 MU/mg and $[\alpha]_D^{25}$ +18.5°. On rechromatography on Whatman No. 1 paper it gave a strong ninhydrin-positive spot at R_F 0.26, and a faint Weber-positive toxin spot at R_F 0.32, indicating the majority of this band to be component 1. The second fraction examined had toxicity 600 MU/mg and $[\alpha]_D^{25}$ +14.5°. On rechromatography on Whatman No. 1 paper it gave a faint Weber-positive toxin spot at R_F 0.32 and a strong ninhydrin-positive spot at R_F 0.38, indicating the majority of this band to be component 3. Judging from the toxicity assays on the two fractions, not more than 10% of each can be toxin. It was therefore concluded that the minor amounts of components 1 and 3 which are present in highly toxic samples recovered from the tailings are not responsible for the low optical rotations observed. It was also established that the low rotations do not owe their origin to an impurity extracted from the paper during recovery of the toxin. Measurements on 1% aqueous solutions of the impurity recovered from seed-test paper in the R_F range 0.14–0.25 exhibited negligible optical activity and in the actual purification of tailings the concentration of the impurity in the recovered toxin is approximately 100-fold more dilute.

Four alternative explanations for the observed rotations are possible. (*a*) Paper chromatography has eliminated some impurity with a rotation higher than +100° which was not removed from clam poison by the methods of Schantz *et al.* (*b*) The toxin present in the tailings is not clam poison but another toxin of closely related structure which has a lower positive rotation. (*c*) The tailings contain two toxins, clam poison and another toxin of lower positive rotation, which are not separated by paper chromatography. (*d*) The tailings contain a non-toxic impurity with a high negative rotation which is not separated from clam poison by paper chromatography.

Alternative (*a*) appears unlikely because Schantz *et al.* (1, 10) obtained clam poison of identical toxicity and rotation (5500±500 MU/mg and $[\alpha]_D^{25}$ +130°±5°) by three different methods of purification, viz. by chromatography on alumina, by chromatography on Norit A followed by crystallization as the helianthate, and by countercurrent distribution. Also, we have observed the same physical constants for clam poison purified by semimicro quantitative paper chromatography (5). It should be emphasized, however, that the chromatographic properties of the clam poison sample purified earlier (5) by the semimicro method and the clam poison tailings purified by the macro method reported herein do not correspond in all respects. It seems reasonable, therefore, to assume that the abnormal optical rotations encountered in the present work owe their origin to some material in the tailings which was absent from the sample purified earlier by the semimicro method and that the nature of the unknown material is explicable by one of the alternatives (*b*), (*c*), or (*d*) mentioned above. Alternative (*b*) at first appears unlikely because of the infrared spectral data. However, the infrared spectrum of clam poison contains little fine structure and may be relatively insensitive to minor structural changes involving, for example, stereoisomerism or tautomerism. In this connection it has been

shown by Schantz *et al.* (10, 13) that little change in the spectrum results from conversion of clam poison to dihydro clam poison by catalytic hydrogenation, even though all the toxicity is lost in the process and the chromatographic properties change markedly (10). Mold *et al.* have obtained evidence from countercurrent distribution studies at pH 8 that clam poison can exist in two tautomeric forms, both of which are highly toxic (10). It may therefore be that the reason for retention of the tailings toxin by the alumina during the original purification is that its structure differs in some subtle manner from clam poison. That minor structural changes can cause significant differences in ease or order of elution of compounds in adsorption chromatography is well known (11). The possibility should also not be overlooked that the toxin recovered from the tailings is a partially degraded clam poison. Dr. E. J. Schantz has recently informed us in a private communication that studies in his laboratory have shown that the optical rotation of clam poison decreases to +50° without concomitant reduction in toxicity by treatment with aqueous alkali under anaerobic conditions. Also, evidence has been obtained by Dr. Schantz and his co-workers that the portion of the molecule responsible for toxicity is not responsible for the optical activity.

If the tailings toxin does differ from clam poison it is possible that the toxicity of the former could be significantly greater than the limits observed by Schantz *et al.* (1) for pure clam poison. Samples of tailings toxin of toxicity 5400±400 MU/mg and $[\alpha]_D^{25}$ +98°±4° were repurified repeatedly by the heavy-paper technique but neither the toxicity nor the specific rotation was significantly increased. It was therefore concluded that either the maximum purity for the material had been attained or that paper chromatography was incapable of effecting further purification. These results make it impossible to differentiate among alternatives (*b*), (*c*), and (*d*) at the present time and an answer to these questions must await further examination of the homogeneity of the tailings toxin by development of other methods of purification.

This work has established the feasibility of enriching and recovering by preparative paper chromatography on seed-test paper a toxin from hitherto intractable residues of low toxicity from the Schantz alumina chromatographic method for purification of clam poison. Residues of bio-assay as low as 300 MU/mg can be enriched by multiple batch treatments. On a 500-mg scale, tailings of toxicity 1400 MU/mg and $[\alpha]_D^{25}$ +26° can be enriched fourfold to 5400±400 MU/mg and $[\alpha]_D^{25}$ +98°±4° in 67% yield by four passes. Further work is required to elaborate the identity of the recovered toxin.

EXPERIMENTAL

Pretreatment of Papers

Whatman No. 17 and seed-test papers were trimmed to 18×22 in. and ½-in. serrations were cut along one 18-in. edge. To the other 18-in. edge was attached a stirrup and two wicks of Whatman 3MM paper (6). Eight such sheets supported by glass rods in a Model A 300 Chromatocab equipped with solvent troughs and reservoirs (6) were washed chromatographically by the procedure described earlier (5) for Whatman No. 1 paper.

Blank Runs on Pretreated Paper

After removal of one wick, the pretreated paper was developed by the descending method with *t*-butanol:acetic acid:water (2:1:1) until the solvent front was 1 to 1½ in. from the serrated edge. This operation required 16 hours for No. 17 paper and 40 hours for seed-test paper. The paper was air-dried at room temperature and rectangular strips were cut from the papers at the R_F ranges specified in Table I. To each strip a stirrup and two wicks of pretreated Whatman No. 1 paper were attached, after which the strips

were eluted with water (20 ml). The eluates were concentrated *in vacuo*, then lyophilized and weighed (5). To determine the quantity of particulate impurity in the residues, the latter were reconstituted in water (3 ml), filtered through a fine-porosity fritted-glass funnel, and the combined filtrate and washings (8 ml) were concentrated, lyophilized, and weighed. To determine the anionic impurity removable from the latter residues, they were reconstituted in water (3 ml) and eluted through Amberlite IRA-400 (Cl) resin (3 ml). The combined eluate and washings (15 ml) were concentrated, lyophilized, and weighed.

Preliminary Purification and Chromatography of Clam Poison Tailings

A sample of clam poison hydrochloride tailings approximately 25% pure was kindly provided for study by the U.S. Army Chemical Research and Development Laboratories. When samples of the aqueous alcoholic solution (14 mg/ml total solids, 18,700 MU/ml, specified by sender) were lyophilized, the total solids found was 14.7 mg/ml and the presence of oily droplets in the lyophilizates, which were water insoluble, was observed. Accordingly, the entire aqueous alcoholic solution was lyophilized and the mixture of oil and solid extracted with 5×2 ml of water to separate the water-soluble material from the oil. The oil and water-soluble fractions were lyophilized after filtration, and bio-assay confirmed that all the toxicity was confined to the water-soluble fraction, which weighed 2.02 g, and had toxicity 1400 MU/mg and $[\alpha]_D^{25}$ +26.3° (1% aqueous solution). Solutions used for chromatography were prepared directly from this material. For chromatograms on pretreated Whatman No. 1 paper, 20 λ of a 1% aqueous solution (200 γ), and on the pretreated heavy papers, 10 λ of a 30% aqueous solution (3 mg), were applied and developed in glass chromatographic tanks by the descending method. The air-dried chromatograms were examined under ultraviolet light and sprayed with Weber reagent (8), Sakaguchi reagent (9), or ninhydrin for location of the components.

Purification of Clam Poison Tailings on Seed-test Paper

A sample of clam poison tailings solution was lyophilized, weighed, and made up to exactly 2 ml in a volumetric flask. Aliquots (0.2 ml) of this solution were applied as a streak 3½ in. long to a strip of pretreated seed-test paper 4¾×22 in., air-drying between each application until the desired quantity of tailings had been applied. The paper was developed by the descending method in a glass chromatographic tank (or in a Model A 300 Chromatocab when 18×22-in. sheets were used) containing *t*-butanol:acetic acid:water (2:1:1) for 40 hours, using a single wick of Whatman 3MM paper. The chromatogram was air-dried and examined under ultraviolet light for location of the components. The bands containing the fluorescent materials were cut from the paper and a stirrup and single wick of pretreated Whatman No. 1 paper was attached to each strip. The strips were eluted with water (20 ml) and the eluates were concentrated *in vacuo* to a volume of 3–5 ml. The concentrates were passed through Amberlite IRA-400 (Cl) resin (3–4 ml), filtered through a fine-porosity fritted-glass disk, concentrated *in vacuo* to a volume of ca. 1 ml, lyophilized, and weighed on the microbalance. From the weights of fractions thus obtained the weights of impurities known to be introduced as a result of blank runs on the same batch of pretreated paper were subtracted to give the corrected weight of each fraction. When side bands from purifications (e.g. fractions 1 and 3, Table III) were reprocessed, the size of the strip of paper to which such material was applied was decreased so that a loading equivalent to 500 mg per 18×22-in. sheet was employed. For bio-assay, solutions of concentration 20 γ/ml were prepared using water of pH 3.00 (hydrochloric acid). Rotations were measured on 1% aqueous solutions.

Bio-assay

Bio-assays were performed on male mice weighing 19–21 g by the method of Schantz *et al.* (12), using ten mice per assay. The conversion factor (C.F. value) for the strain of mice used in these experiments was determined using a standard sample of clam poison provided by Dr. Schantz and was found to be 0.160 γ per MU.

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THE FRACTIONATION OF POLYTHENE[1]

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ABSTRACT

An apparatus is described for fractionating large quantities (400 g) of polythene into five roughly equal fractions using a fractional precipitation technique. Application of this method of fractionation to a linear polythene has shown that the width of the molecular-weight distribution of the successive fractions decreases as the fractionation proceeds. Consequently, the initial high-molecular-weight fractions require refractionation to produce equally narrow distributions in them as are found in later fractions. Good agreement is obtained with the experimentally determined values of the number-average and weight-average molecular weight for the parent polymer when the measured values of M_n and M_w for each fraction are used to calculate the values for the parent. The differential molecular-weight distribution function of the parent polymer was calculated on a Bendix G-15 computer from the data for the fractions by using the weight, number-average and weight-average molecular weight, measured for each fraction in conjunction with an assumed log-normal or negative binomial molecular-weight distribution function in each fraction.

INTRODUCTION

The preparation of polythene samples with narrow molecular-weight distributions is more conveniently done by fractionation than by synthesis. Two techniques are most commonly used, fractional solution and fractional precipitation. Both methods appear to be equally efficient when used in dilute solution at or above the melting point of the polymer (1). The present study used a precipitation technique in which the polymer was dissolved in a suitable solvent (*p*-xylene) and then partially precipitated by adding a non-solvent (triethylene glycol) (2). In this way, a large-scale fractionator was used to divide a 400-g polymer charge into five 80-g fractions. The fractions were then stored until enough material had been collected in each fraction to warrant refractionation.

EXPERIMENTAL

Apparatus

The large-scale fractionator consisted of an upper borosilicate glass separator vessel of 50-liter capacity and a lower 40-liter metal storage tank, both immersed in a 200-gal, totally enclosed oil bath maintained at an operating temperature of 130° C by six thermistor-controlled 1000-w electric heaters. The temperature of the bath was measured by means of six precision thermometers placed at different locations in the bath. Temperature fluctuations were held to within ±0.05° C of the operating temperature throughout the entire bath by employing a highly efficient stirring arrangement and by using two specially designed ultrasensitive thermistor controllers (3) to control the temperature. The bath fluid, a water-white mineral oil maintained under an atmosphere of CO_2 to prevent oxidation, could be used for 2 months before it darkened and had to be discarded. Phase boundaries in the separator vessel were illuminated by means of three strategically placed, sealed-beam automobile head lamps submerged in the oil bath, and could be viewed through several glass observation ports in the side of the bath. The solvent, *p*-xylene, and the non-solvent, triethylene glycol, were separately preheated to 130° C in steam-jacketed, glass-lined kettles which were connected by steam-traced lines to the separator vessel. A specially designed burette was used to determine the quantities of hot triethylene glycol added to the separator. The layout of the apparatus is shown in Fig. 1. The selection

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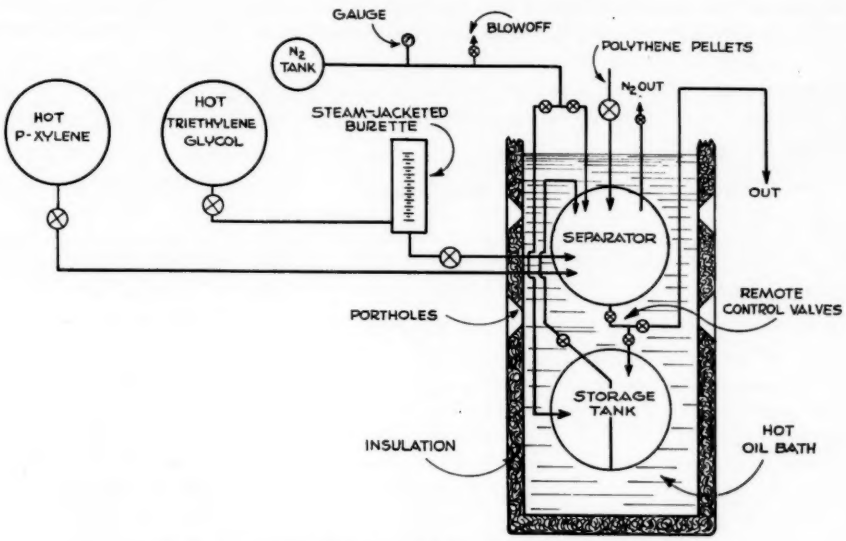

FIG. 1. Apparatus for large-scale fractionation of polythene.

of the operating temperature was determined by the melting point of the polymer and was chosen so that the precipitated material would be liquid and crystallization would not interfere with the equilibrium distribution of polymer between the two phases.

Procedure

The polymer was fractionated by partial precipitation in a manner similar to that described by Tung (2). A 1.6% solution (w/v) of the polythene in *p*-xylene (containing 0.1% w/v 2,6-ditertiary-butyl-*p*-cresol as antioxidant) was vigorously stirred under dry nitrogen in the separator and the non-solvent, triethylene glycol, slowly added until the first signs of opacity appeared in the solution. Sufficient non-solvent (determined by trial and error) was then added to precipitate out approximately 20% (80 g) of the total charge of polythene in the solution. After a period of vigorous agitation followed by a settling period, the upper gel phase was separated from the lower solution in the following manner: the lower solution was drained into the storage tank, then enough fresh *p*-xylene was added to form a clear solution of the gel phase, finally the gel was redissolved, and the fresh solution was siphoned out of the separator and into 10 gal of vigorously stirred cold methanol.

The solution in the storage tank was then transferred to the separator vessel by applying nitrogen pressure to the storage tank. Subsequent fractions were then precipitated by following the same procedure. In general the first fraction required 3 hours to settle; however, later fractions required longer settling times, fraction 4 requiring at least 12 hours. The fifth fraction was obtained by siphoning off the solution from the gel phase of fraction 4 and adding it directly to cold methanol. Each 80-g fraction was filtered off and then washed five times with cold methanol before being transferred to a special washing vessel in which the filtrate was stirred in contact with cold methanol (containing 0.05% antioxidant) for at least 24 hours before being filtered off and dried to constant weight in a vacuum oven. The yield efficiency of these partial precipitations was found

to be about 95%. Studies indicated that polymer losses in filtration and washing amounted to 2.5%, leaving a further 2.5%, or 10 g, of polymer unaccounted for. This loss may have been due to low-molecular-weight material present in the polythene dissolving in the cold methanol/xylene mixture. However, cooling the methanol/xylene mixture to −72° C and allowing the material to warm up slowly until the xylene crystals melted failed to yield any precipitate.

DISCUSSION

Results

As an indication of the extent of fractionation actually achieved with the above system, measurements on fractions obtained from a linear polythene are given in Table I.

TABLE I

Typical fractionation results with a linear polythene

Run	Fraction	Cumulative wt. (g)	Cumulative % recovered	M_n (Me/1000)	M_n (vinyl)	η (120° C, tetralin)	Calc. M_w	M_w/M_n (vinyl)
4	1	63.4	15.8	33,300	29,700	3.70	240,000	8.1
	2	138.4	34.6	24,900	33,200	2.27	128,000	3.9
	3	237.4	59.3	30,800	29,200	1.22	58,000	2.0
	4	319.5	79.8	14,600	16,900	0.70	28,500	1.7
	5	382.0	95.5	2,400	3,800	0.28	8,800	2.3
Parent polymer as measured:				12,900	11,000	1.71	90,000	8.2
Calculated for the parent from the measured values for the fractions:					13,100		87,600	8.2

Columns 5 and 6 list number-average weights (M_n) for each fraction, based on the assumption that each polymer molecule is terminated by a methyl group at one end and a vinyl group at the other. Both groups were measured by infrared spectroscopy, to within an accuracy of 20% in the methyl group determination (4) and 5% in the case of the vinyl group determination. Vinyl group measurements were based on the change in intensity of the 908 cm^{-1} band which arises when the sample is brominated (5). The weight-average molecular weights (M_w) were calculated from the intrinsic viscosities (η) for each fraction using the Duch and Kuchler relationship (7). The intrinsic viscosities were measured at 120° C in tetralin solutions using Schulken and Sparks (6) viscometers. The good agreement between (M_n) values determined by the two methods verifies the assumption that the molecule is terminated by a methyl and a vinyl group. The decreasing values of the ratios of the molecular weights, M_w/M_n, indicate that the width of the distribution in each consecutive fraction decreases and that refractionation is necessary if fractions of equally narrow molecular-weight distribution are required. Cragg and Hammerschlag (1) have pointed out that refractionation more than thrice is inefficient and this number of refractionations has been accepted as the limit. The value of the number-average molecular weight for the parent polymer was calculated using the measured number-average molecular weights of the fractions and the formula $M_n = \sum n_i m_i / \sum n_i$ and showed good agreement with the experimentally determined value for the unfractionated polymer (Table I). Likewise, the weight-average molecular weight of the parent polymer calculated from the expression $M_w = \sum m_i w_i / \sum w_i$ also showed good agreement with the value calculated from the measured intrinsic viscosity of the

unfractionated polymer. This agreement suggests that the parent polymer had not been chemically changed by the fractionation procedure.

Calculation of the Molecular-Weight-Distribution Curves

Pre-1939 molecular-weight-distribution curves were obtained by making some estimate of the molecular weight of each fraction and then plotting the cumulative weight of the first n fractions against the estimated molecular weight of the nth fraction to yield the integral weight-distribution curve. This procedure assumed incorrectly that there was no overlap between fractions and also that every species present in the nth fraction had a molecular weight between the average measured for the $(n-1)$th and nth fractions. A somewhat more realistic approach was made by Schulz (8), who assumed the fraction to be symmetrically distributed about its average molecular weight. In this calculation, the total weight of the first $(n-1)$ fractions plus half the weight of the nth fraction is plotted against the molecular weight of the nth fraction; however, this still neglects the overlapping between fractions.

In the last decade, serious attempts have been made to allow for the overlap of the fractions by assuming a differential distribution function for each fraction, summing these functions to obtain the differential distribution curve for the parent polymer, and then forming the integral distribution curve (9, 10, 11). More recently two independent studies (12, 13) have suggested that the most satisfactory results are obtained when the amount of each fraction and the number- and weight-average molecular weight are also known. The differential distribution function for the parent polymer may then be calculated using these three parameters and assuming a relatively simple form for the distribution functions of the fractions. A program for the calculation of the molecular-weight distribution of the parent polymer was written for the Bendix G-15 computer, given the three parameters and assuming either a log-normal or a negative binomial distribution for the fractions. Results are given in Table I.

ACKNOWLEDGMENTS

The authors wish to acknowledge discussions of this work with Mr. A. M. Birks; to thank Dr. D. C. West for having programed the calculation of the polymer molecular-weight-distribution function on the Bendix computer; and to thank Mr. T. Finn, who performed most of the experimental work. The authors also wish to thank Mr. A. W. Pross for the infrared measurements and Dr. H. P. Schreiber for the intrinsic viscosity results.

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THE GAS–LIQUID PARTITION CHROMATOGRAPHY OF CARBOHYDRATE DERIVATIVES

PART I. THE SEPARATION OF GLYCITOL AND GLYCOSE ACETATES[1]

S. W. GUNNER, J. K. N. JONES, AND M. B. PERRY

ABSTRACT

Gas–liquid partition chromatography has been used to separate the fully acetylated derivatives of tetritols, pentitols, hexitols, heptitols, octitols, and anomeric glycoses.

DISCUSSION

Gas–liquid partition chromatography (G.L.P.C.) has already been shown to be a powerful technique for the preparative separation and for the qualitative and quantitative analysis of carbohydrate derivatives. Bishop and his co-workers (1, 2) were the first to report the separation of methyl *O*-methyl glycosides by G.L.P.C. and have since demonstrated the use of such separations in the analysis of these derivatives obtained from the fission of methylated polysaccharides (3, 4, 5). Kircher (6) has also described the G.L.P.C. separation of methylated methyl glycosides employing other types of liquid phases. *O*-Methyl aldonolactones (7), *O*-methyl-*O*-acetyl glycitols (8), *O*-acetyl glycitols (9), and other carbohydrate derivatives (10) have also been successfully resolved using the G.L.P.C. technique.

In view of its potential use in the field of carbohydrate chemistry, a systematic program was undertaken in this laboratory to explore the conditions under which favorable separations of a variety of carbohydrate derivatives might be undertaken using G.L.P.C. This paper records the results obtained in investigations into the separation of glycitol and glycose acetates. Until recently the methods available for the separation of acetylated glycitols and glycoses involved column chromatography on a Magnesol–Celite or Silene EF – Celite (11*a*) mixture using benzene – *tert*-butyl alcohol as the mobile phase, cellulose column chromatography, or paper chromatography (11*b*). It is considered that the G.L.P.C. method of separation will provide a more rapid and refined procedure.

The recorded separations were carried out using samples of the acetate derivatives (2–5 γ) developed on a Pye Gas Argon Chromatograph fitted with an ionization detector (12), and in cases where larger amounts of material (20–200 mg) were used for preparative work, the Burrell Kromo-Tog, Model K-2 apparatus was used. Experience with other types of chromatographic equipment showed that it was necessary for the sample to be injected directly onto the column packing material in order to prevent the decomposition of the samples, which was observed when a dead space existed between the injection site and the beginning of the column packing material. In this connection it is important to note that small gaps in the packing material itself should be avoided and that the detector should be placed as close as possible to the end of column packing for satisfactory results.

Acid-washed Chromosorb W or silver-coated Chromosorb W proved to be a suitable inert support which did not show excessive adsorption of the acetate derivatives. The non-polar liquid phases chosen were Apiezon M vacuum grease or Dow Corning stopcock grease, and the polar phase selected was butanediol succinate polyester. Columns prepared containing 20% w/w of the non-polar liquid phases gave fair separations of the

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Contribution from the Department of Organic Chemistry, Queen's University, Kingston, Ontario. Abstracted in part from the M.Sc. Thesis of S. W. Gunner, Queen's University, 1961.

acetate derivatives with only slight adsorption-tailing effects. Columns prepared using 20% w/w of the polar phase on Chromosorb W gave increased resolution of the derivatives but had the disadvantage of markedly increasing their retention times. It was considered that mixtures of the packing materials made up of a non-polar and polar liquid phase component might combine the advantages of each individual liquid phase to give a column packing which would have a high resolving power and would give reasonable retention times for the glycitol and glycose acetates. In practice it was found that the ideal combinations were (*a*) a 1:1 w/w mixture of 20% w/w Apiezon M grease on silver-coated Chromosorb W and 20% w/w butanediol succinate polyester on Chromosorb W (column packing A) and (*b*) a 1:1 w/w mixture of 20% w/w Dow Corning grease on Chromosorb W and 20% w/w butanediol succinate polyester on Chromosorb W (column packing B).

In attempts to find a column packing material suitable for the separation of oligosaccharide acetates (13), H. G. Jones, in this laboratory, developed a packing material of 0.1 to 0.3% Apiezon M vacuum grease on silver-coated glass beads (column packing D). It was found that the retention times for monosaccharide acetates on this column were extremely short. The previously mentioned mixed column packings (A and B) gave satisfactory separations for the glycitol and glycose acetates up to those derived from the C_6 compounds but those derivatives of higher molecular weight showed considerable broadening of the peaks with increasing retention times. This latter difficulty was overcome by using a column made from a 1:1 v/v mixture of column packings A and D (column packing C), which gave satisfactory separations of the acetates derived from the C_7 and C_8 glycitols and glycoses, as well as retaining the ability to resolve the lower molecular weight derivatives. The glass bead column (column packing D) provided a rapid separation of the acetates derived from C_4 to C_8 glycitols in order of their increasing molecular weights, but under the conditions used it did not separate the individual glycitol acetates having the same molecular weights.

Typical separations of the glycitol acetates on column packing A are shown in Fig. 1, on column packing C in Fig. 2, and on column packing D in Fig. 3. Typical separations of anomeric glycose acetates on column packing A are shown in Fig. 4.

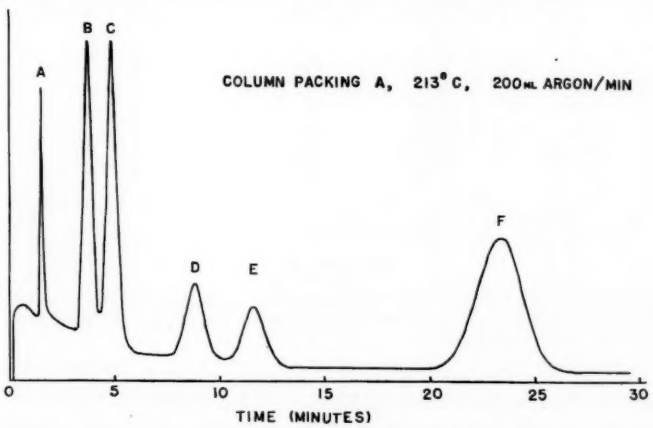


FIG. 1. Separation of glycitol acetates: (A) tetra-*O*-acetylerythritol, (B) penta-*O*-acetylribitol, (C) penta-*O*-acetylxylitol, (D) hexa-*O*-acetylallitol, (E) hexa-*O*-acetyl-D-iditol, and (F) hepta-*O*-acetyl-D-*glycero*-D-galactoheptitol.

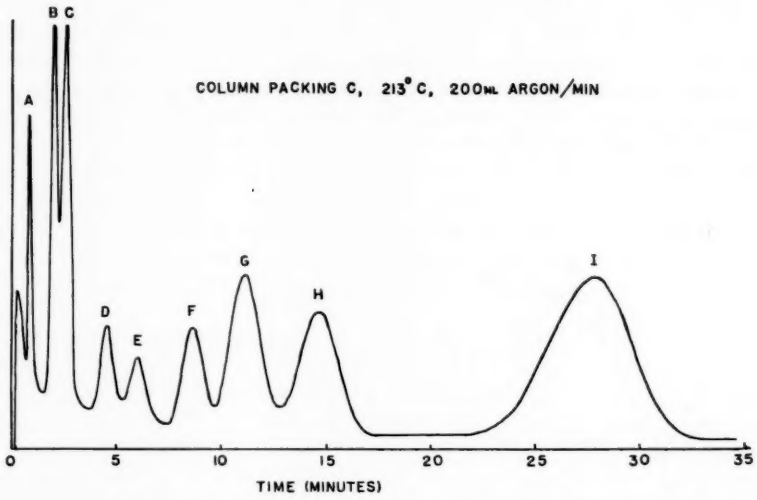


FIG. 2. Separation of glycitol acetates: (A) tetra-*O*-acetylerythritol, (B) penta-*O*-acetylribitol, (C) penta-*O*-acetylxylitol, (D) hexa-*O*-acetylallitol, (E) hexa-*O*-acetyl-D-iditol, (F) hepta-*O*-acetyl-meso-*glycero*-alloheptitol, (G) hepta-*O*-acetyl-D-*glycero*-D-mannoheptitol, (H) hepta-*O*-acetyl-L-*glycero*-D-glucoheptitol, and (I) octa-*O*-acetyl-D-*erythro*-L-galactooctitol.

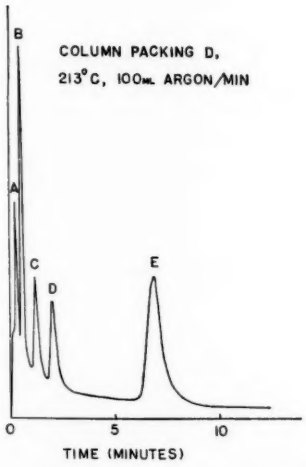


FIG. 3. Separation of glycitol acetates: (A) penta-*O*-acetyl-L-arabitol, (B) hexa-*O*-acetyl-D-glucitol, (C) hepta-*O*-acetyl-D-*glycero*-D-galactoheptitol, (D) octa-*O*-acetyl-D-*threo*-L-galactooctitol, and (E) hepta-*O*-acetyl 5-*O*-β-D-xylopyranosyl-L-arabitol.

Table I records the retention times of the glycitol acetates relative to penta-*O*-acetyl-L-arabitol (= 1.00) on all four column packing materials. Table II records the retention times of glycose acetates relative to penta-*O*-acetyl-L-arabitol (= 1.00) on column packing materials A, B, and C.

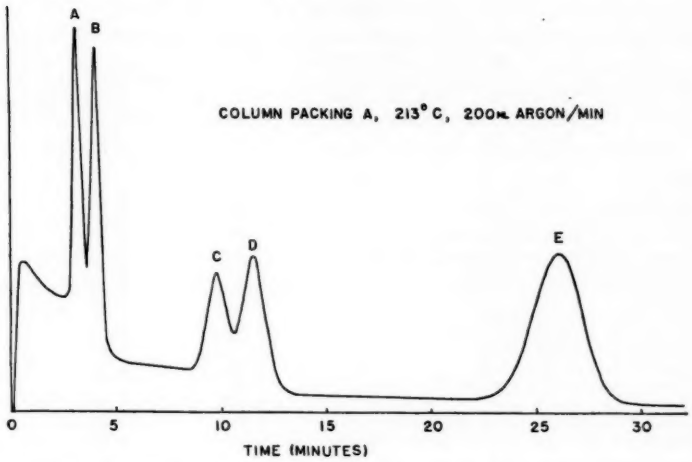


FIG. 4. Separation of anomeric glycose acetates: (A) tetra-*O*-acetyl-α-D-xylopyranose, (B) tetra-*O*-acetyl-β-D-xylopyranose, (C) penta-*O*-acetyl-α-D-glucopyranose, (D) penta-*O*-acetyl-β-D-glucopyranose, and (E) hexa-*O*-acetyl-D-*glycero*-α-D-glucoheptopyranose.

TABLE I

Retention times of fully acetylated glycitols relative to penta-*O*-acetyl-L-arabitol at 213° C

Glycitol acetate	Column packing material			
	A	B	C	D
Glycerol	0.120	0.118	0.122	—
Erythritol	0.378	0.358	0.393	—
D-Threitol	0.403	0.429	0.433	—
Ribitol	0.918	0.895	0.944	—
L-Arabitol	1.000	1.000	1.000	1.000
Xylitol	1.18	1.18	1.20	—
Allitol	1.78	1.89	1.90	—
D-Talitol	2.07	2.16	2.03	—
D-Mannitol	2.13	2.25	2.16	—
D-Glucitol	2.39	2.59	2.73	1.85
Galactitol	2.42	2.59	2.75	—
D-Iditol	2.74	2.97	2.85	—
6-Deoxy-L-talitol	0.707	0.712	0.698	—
L-Rhamnitol	0.715	0.733	0.730	—
D-Fucitol	0.750	0.778	0.750	—
Pentaerythritol	0.807	0.798	0.790	—
meso-*Glycero*-alloheptitol	—	—	3.56	—
D-*Glycero*-D-altroheptitol	—	—	4.06	—
D-*Glycero*-D-mannoheptitol	—	—	4.73	—
meso-*Glycero*-guloheptitol	—	—	4.90	—
D-*Glycero*-D-galactoheptitol	5.60	—	5.05	3.92
D-*Glycero*-D-glucoheptitol	—	—	5.10	—
L-*Glycero*-D-glucoheptitol	—	—	6.11	—
meso-*Glycero*-idoheptitol	—	—	6.60	—
D-*Erythro*-L-galactooctitol	—	—	11.50	—
D-*Threo*-L-galactooctitol	—	—	12.61	6.52
5-*O*-β-D-Xylopyranosyl-L-arabitol	—	—	—	22.3

These results show that by using column packings A, B, and C, well-defined separations of the two tetritol derivatives, tetra-*O*-acetylerythritol and tetra-*O*-acetyl-D-threitol, and

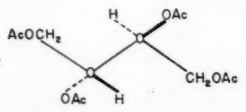
TABLE II

Retention times of glycose acetates and acetylated methyl glycosides relative to penta-*O*-acetyl-L-arabitol at 213° C

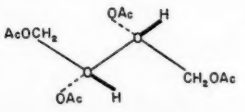
	Column packing material		
	A	B	C
Glycose acetates			
Tetra-*O*-acetyl-α-D-xylopyranose	0.715	0.700	0.605
Tetra-*O*-acetyl-β-D-xylopyranose	0.910	0.891	0.778
Penta-*O*-acetyl-α-D-galactopyranose	2.04	2.05	1.92
Penta-*O*-acetyl-β-D-galactopyranose	2.70	2.258	2.55
Penta-*O*-acetyl-α-D-glucopyranose	2.14	2.25	2.21
Penta-*O*-acetyl-β-D-glucopyranose	2.57	2.57	2.41
Penta-*O*-acetyl-α-D-mannopyranose	2.22	2.35	2.26
Penta-*O*-acetyl-β-D-mannopyranose	2.74	2.73	2.64
Tetra-*O*-acetyl-α-D-ribopyranose	1.00	1.07	0.988
Tetra-*O*-acetyl-α-D-ribofuranose	1.12	1.21	1.13
Penta-*O*-acetyl-α-D-altropyranose	2.52	2.69	2.50
Penta-*O*-acetyl-α-L-lyxose	0.842	0.802	0.800
Hexa-*O*-acetyl-D-*glycero*-α-D-mannoheptopyranose	4.96	4.93	4.93
Hexa-*O*-acetyl-D-*glycero*-α-D-glucoheptopyranose	5.60	5.63	5.60
Acetylated methyl glycosides			
Methyl tri-*O*-acetyl-α-L-rhamnopyranoside	0.291	0.293	0.310
Methyl tetra-*O*-acetyl-β-D-glucopyranoside	1.48	1.45	1.40

the three pentitol derivatives, penta-*O*-acetylribitol, penta-*O*-acetyl-L-arabitol, and penta-*O*-acetylxylitol, were obtained. All the hexitol acetate derivatives, with the exception of hexa-*O*-acetyl-D-glucitol and hexa-*O*-acetylgalactitol could be separated from each other. All the heptitol acetate derivatives could be separated from each other as well as the two octitol acetate derivatives which were examined. The order of emergence of the glycitol acetates was found to be independent of the nature of the column packing materials used, although some differences in the relative retention times for the compounds were observed. It was also found that the same order of emergence of the compounds was retained using column temperatures ranging from 200° to 250° C. At 250° the retention times for the glycitol acetates were very much decreased, and sharper peaks than those recorded in Figs. 1, 2, and 3 were obtained. No decomposition of the derivatives was detected using column temperatures up to 250° and the derivatives were recovered unchanged from preparative-scale separations.

The results collected so far indicate that a relationship exists between the retention times of the glycitol acetates and their stereochemical structure described in the conventional zig-zag conformations. The retention times of the glycitol acetates having the same molecular weight depends on the arrangement of acetoxy groups attached to the non-terminal carbon atoms. The greater the number of these acetoxy groups which are arranged on one side of the molecule, the greater is the affinity of the compound for the liquid phase and hence the greater its retention time. Thus, tetra-*O*-acetylerythritol (I) has a shorter retention time than tetra-*O*-acetyl-D-threitol (II), which has the two acetoxy groups attached to the non-terminal carbon atoms arranged on one side of the molecule.

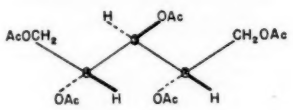


(I)

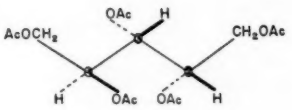


(II)

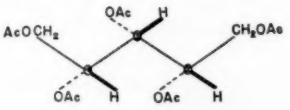
In the case of the three pentitol acetate derivatives, both penta-*O*-acetylribitol (III) and penta-*O*-acetyl-L-arabitol (IV) have a maximum of two acetoxy groups attached to non-terminal carbon atoms arranged on one side of the molecule. It would appear that in this case as well as the case of hexitol acetate and heptitol acetate derivatives, where there are two or more glycitol acetates of the same molecular weight and having the same maximum number of acetoxy groups arranged on one side of the molecule, the closer these acetoxy groups are to each other the greater is the affinity of the compound for the liquid phase and hence the greater its retention time. Thus, penta-*O*-acetylribitol (III) has a lower retention time than penta-*O*-acetyl-L-arabitol (IV). Penta-*O*-acetylxylitol (V), with the maximum of three acetoxy groups attached to non-terminal carbon atoms arranged on one side of the molecule, has the longest retention time of the three pentitol acetate derivatives.



(III)

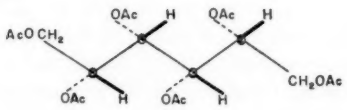


(IV)

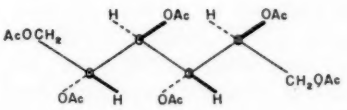


(V)

On the basis of the empirical rules set out above it would be predicted that hexa-*O*-acetyl-D-iditol (VI), which has all four of its acetoxy groups attached to non-terminal carbon atoms arranged on one side of the molecule, should be the hexitol acetate derivative having the longest retention time, and hexa-*O*-acetylallitol (VII), which has a maximum of two acetoxy groups attached to non-terminal carbon atoms arranged on one side of the molecule, and having the maximum possible separation, should be the hexitol acetate derivative having the shortest retention time. This prediction has proved correct in practice.

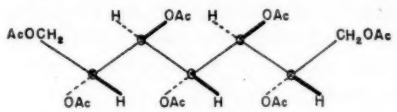


(VI)

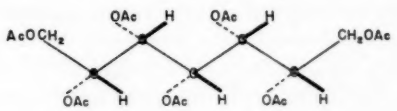


(VII)

In agreement with the above statements, it was found that hepta-*O*-acetyl meso-*glycero*-alloheptitol (VIII), which has a maximum of three acetoxy groups attached to non-terminal carbon atoms arranged on one side of the molecule and having the maximum possible separation, has the shortest retention time of all the heptitol acetate derivatives, whereas hepta-*O*-acetyl-meso-*glycero*-idoheptitol (IX), which has all the acetoxy groups attached to the non-terminal carbon atoms arranged on one side of the molecule, has the longest retention time. The chromatographic behavior of the glycitol acetate derivatives having relative retention times between the extremes cited above is in agreement with



(VIII)



(IX)

that predicted from a consideration of the spatial distribution of the acetoxy groups attached to the non-terminal carbon atoms.

The acetate derivatives of the deoxyglycitols, 6-deoxy-L-talitol, L-rhamnitol, and D-fucitol, can be separated by G.L.P.C., and their order of elution from the columns used is the same as those of their parent hexitol derivatives (L-talitol, L-mannitol, and galactitol).

The anomeric acetates of D-xylopyranose, D-galactopyranose, D-glucopyranose, and D-mannopyranose were separated by G.L.P.C. as were the tetra-*O*-acetyl derivatives of α-D-ribopyranose and α-D-ribofuranose. It is probable that the retention times for these derivatives depend upon the relative positions of the axial and equatorial *O*-acetyl groups but with the limited results available at this time it is not possible to rationalize their chromatographic behavior.

G.L.P.C. has also been shown to afford a method for the separation of acetylated glycosides.

EXPERIMENTAL

Apparatus

The recorded separations were carried out using a Pye Argon Chromatograph fitted with an ionization detector (80 μc radium D) and employing straight glass columns (120×0.5-cm I.D.) packed with the appropriate support enclosed at each end by small glass wool plugs. The samples (2–5 γ) in dry chloroform solution were placed on the top of the column packing using a micropipette and development was made using dry argon as the carrier gas.

Preparative-scale separations (20–200 mg) were made on U-shaped glass columns (255×1.3-cm I.D.) filled with the support material, using the Burrell Kromo-Tog, Model K-2 apparatus fitted with a thermal detector and employing helium as the carrier gas.

Column Packing

Chromosorb W (Johns-Manville), 60–80 mesh, was washed with 2 *N* hydrochloric acid and then with distilled water until the washings were neutral, was dried at 110°, and was then screened to remove fines. Silver-coated Chromosorb W was prepared by treating acid-washed Chromosorb W (60–80 mesh) (10 g) with 120 ml each of two solutions A and B. Solution A was prepared by just adding sufficient dilute ammonium hydroxide solution to a solution of silver nitrate (15.8 g) in water (300 ml) to produce a clear solution. Solution B was prepared by adding a solution of Rochelle salt ($NaKC_4H_4O_6$, 2.5 g) in water (25 ml) to a boiling solution of silver nitrate (3 g) in distilled water (350 ml) and, after filtration, the volume of the solution was adjusted to 350 ml. After treatment of the stirred Chromosorb W with the first two portions of solutions A and B the supernatant was removed after 10 minutes, and the residue was further treated with the same amounts of these two solutions. The resulting silver-coated Chromosorb was filtered off, washed with distilled water followed by acetone, and finally dried in an oven at 140°. The weight of silver deposited was 3 g.

Silver-coated glass beads were prepared in a manner similar to that used in the preparation of silver-coated Chromosorb by treating glass beads (60 plus mesh; MS-H; Microbeads Inc., Jackson, Mississippi) (500 g), previously washed with dilute nitric acid and distilled water, with the same quantities of solutions A and B.

The following liquid phases were used: (*a*) Apiezon M vacuum grease (Fisher Scientific Company), (*b*) Dow Corning stopcock grease (Fisher Scientific Company), and (*c*)

butanediol succinate polyester (Craig) prepared according to the directions of Bishop *et al.* (2). The packing materials were prepared by stirring a slurry of a weighed amount of the liquid phase dissolved in chloroform with a weighed amount of the inert support in an open beaker at room temperature while the bulk of the solvent was removed by aeration. The last traces of the solvent were removed by heating the material *in vacuo* at 140°.

The packing materials prepared are described below.

(1) Column packing A.—1:1 w/w mixture of (*a*) 20% w/w butanediol succinate polyester on 60–80 mesh Chromosorb W and (*b*) 20% w/w Apiezon M grease on 60–80 mesh silver-coated Chromosorb W.

(2) Column packing B.—1:1 w/w mixture of 20% w/w butanediol succinate polyester on 60–80 mesh Chromosorb W and (*b*) 20% w/w Dow Corning grease on 60–80 mesh Chromosorb W.

(3) Column packing C.—1:1 v/v mixture of (*a*) column packing A and (*b*) column packing D.

(4) Column packing D.—0.3% w/w Apiezon M grease on 60 plus mesh silver-coated glass beads.

The dried column packing materials were run into the glass columns under gravity with gentle tapping to ensure even packing and the absence of any cavities. The packed columns were purged for 3 hours at 240° with dry nitrogen gas before use. It was found that the columns could be used for several hundred analyses at 213° C before any significant deterioration in their resolving power became apparent.

ACKNOWLEDGMENTS

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FREE RADICALS BY MASS SPECTROMETRY

XXVI. PRIMARY STEPS IN THE MERCURY-PHOTOSENSITIZED DECOMPOSITION OF METHYL ACETATE[1]

R. F. POTTIE[2] AND F. P. LOSSING

ABSTRACT

The $Hg(^3P_1)$-photosensitized reaction of methyl acetate at micron pressures in the presence of 8 mm of helium has been examined in a flow system by mass spectrometry. At 55° C the primary dissociation is mainly into acetyl and methoxy radicals. An alternative dissociation into CO_2 and two CH_3 radicals occurs with a frequency of about one fifth of the principal reaction. A small amount of $COOCH_3$ radical is also formed. No CH_3CO_3 radicals could be found. Since the yield of H_2 is less than 2% of the methyl acetate decomposed, a primary dissociation to give H atoms and CH_3COOCH_2 or CH_2COOCH_3 radicals is quite small.

INTRODUCTION

The photolysis of methyl acetate in the wavelength region 2200–2600 Å was investigated some years ago by Roth and Rollefson (1). From the products of the photolysis at 20° C and 14 mm pressure they concluded that a primary dissociation into acetyl and methoxy radicals accounted for about 95% of the total reaction. A dissociation into CO_2 and, presumably, two methyl radicals accounted for the remainder. They also examined the $Hg(^3P_1)$-photosensitized decomposition under similar conditions and found that the decomposition proceeded by essentially the same paths. The direct photolysis has been investigated in further detail by Wijnen (2, 3, 4), and briefly by Ausloos (5). Wijnen found, in agreement with the earlier work, that the main primary dissociation was to form acetyl and methoxy radicals. A dissociation to give CO_2 also occurred, but to a larger extent than found by Roth and Rollefson. As will be discussed below, Wijnen's data lead to a ratio of about 4:1 for the two modes of dissociation at 30° C.

In the present work, the relative importance of the primary dissociation steps in the mercury-photosensitized decomposition has been reinvestigated.

EXPERIMENTAL

The $Hg(^3P_1)$-photosensitized reactions were carried out using a fast-flow system coupled to a mass spectrometer. This apparatus and its characteristics have been described in earlier publications in this series (6, 7, 8). The reactant and mercury vapor at partial pressures of a few microns were carried in a stream of helium at 8 mm through a tubular fused-silica reaction cell strongly illuminated by a low-pressure mercury lamp of high intensity. A representative portion of the stream entered the ionization chamber of a mass spectrometer through a small orifice at the end of the illuminated zone. The temperature of the cell was maintained at 55° C by circulation of water from a thermostatted bath. The contact time in the illuminated zone was about 0.001 second. The intensity of 2537-Å radiation absorbed was sufficient to decompose acetone, for example, to the extent of about 22% in this period. In effect, the reaction conditions resemble those of flash photolysis, since radical–radical reactions greatly predominate over radical–substrate reactions (8).

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[2]*National Research Council Postdoctorate Fellow 1958–60.*

RESULTS AND DISCUSSION

The yields of products in the mercury-photosensitized decomposition of methyl acetate are given in Table I. The yield of H_2 was less than 2% of the methyl acetate decomposed.

TABLE I

Hg-Photosensitized decomposition of methyl acetate at 55° in 8 mm of helium (partial pressure in microns)

	Run No.			
	1	2	3	4
CH_3COOCH_3				
Lamp off	5.64	5.56	10.27	10.82
Lamp on	4.78	4.54	9.08	9.30
Decomposed	0.86	1.03	1.19	1.51
% decomposed	15.2	18.5	11.6	14.0
$(CH_3CO)_2$	0.030	0.038	0.057	0.078
CH_3COCH_3	0.073	0.093	0.092	0.148
CH_3CHO	0.041	0.061	0.075	0.102
$CH_2{=}CO$	0.131	0.127	0.157	0.177
$(CH_3O)_2CH_2$	0.006	0.005	0.015	0.015
$HCOOCH_3$	0.008	0.017	0.017	0.017
CH_3OCH_3	0.047	0.061	0.064	0.091
CH_3OH	0.189	0.231	0.262	0.326
CH_2O	0.198	0.244	0.279	0.369
C_2H_6	0.073	0.086	0.093	0.144
CH_4	0.074	0.095	0.097	0.138
CH_3	0.171	0.116	0.140	0.122
CO_2	0.122	0.125	0.131	0.154
CO	0.258	0.327	0.303	0.367
C bal., %	73	74	72	73
O bal., %	74	74	70	70
H bal., %	74	71	71	74
R_{CH_3O}, %	50	53	53	54
R_{CH_3CO}, %	34	35	37	39
Total reaction [1], %	59	60	58	58
Total reactions [2] to [4], %	15	14	12	11

In addition to the products listed, a considerable amount of a polymeric substance was formed, which deposited on the walls of the reactor as a yellowish-brown stain. The formation of this material is presumably responsible for the low carbon, oxygen, and hydrogen balances. The low mass balances cannot be attributed to the formation of polymers of formaldehyde. In the photosensitized decomposition of dimethyl ether the carbon and oxygen balances were 95%, although large amounts of formaldehyde were formed (9). Moreover, the near equality of the carbon, oxygen, and hydrogen balances in Table I indicates that the material unaccounted for must have a C:O:H ratio essentially the same as in methyl acetate itself. Since some 25% of the methyl acetate disappears to form an unidentified and presumably non-volatile product, the conclusions reached as to the relative importance of various primary modes of dissociation are necessarily approximate.

The variations in the percentage of methyl acetate decomposed in the four runs in Table I result from the difficulties encountered in keeping the reactor walls reasonably free from opaque deposits.

The presence of substantial yields of products such as $(CH_3CO)_2$, CH_3COCH_3, and CH_3CHO indicates that acetyl radicals are formed in abundance in the decomposition. The corresponding fragment, CH_3O, is clearly the precursor of such products as CH_3OCH_2,

CH_3OH, and CH_2O. The formation of CO_2 and $HCOOCH_3$, on the other hand, must result from an alternative primary step in which a CH_3 radical or possibly two CH_3 radicals are lost from the methyl acetate molecule. Evidently two, and possibly four, primary decomposition steps must be taken into account:

$$CH_3\overset{\overset{O}{\|}}{C}OCH_3 + Hg^* \rightarrow CH_3CO + OCH_3 + Hg \quad [1]$$

$$CH_3\overset{\overset{O}{\|}}{C}OCH_3 + Hg^* \rightarrow CH_3 + \overset{\overset{O}{\|}}{C}OCH_3 + Hg \quad [2]$$

$$CH_3\overset{\overset{O}{\|}}{C}OCH_3 + Hg^* \rightarrow CH_3\overset{\overset{O}{\|}}{C}O + CH_3 + Hg. \quad [3]$$

If the CH_3CO_2 or $COOCH_3$ radicals decompose almost immediately, reactions [2] and [3] will be equivalent to the following reaction:

$$CH_3\overset{\overset{O}{\|}}{C}OCH_3 + Hg^* \rightarrow 2CH_3 + CO_2 + Hg. \quad [4]$$

The secondary reactions of radicals formed in these steps will be (*a*) from reaction [1]:

$$2CH_3CO \rightarrow (CH_3CO)_2 \quad [5]$$

$$2CH_3CO \rightarrow CH_3CHO + CH_2{=}CO \quad [6]$$

$$CH_3CO \rightarrow CH_3 + CO \quad [7]$$

$$CH_3CO + Hg^* \rightarrow CH_2{=}CO + H + Hg \quad [8]$$

$$CH_3CO + CH_3 \rightarrow CH_3COCH_3 \quad [9]$$

$$CH_3CO + CH_3 \rightarrow CH_2{=}CO + CH_4 \quad [10]$$

$$2CH_3O \rightarrow CH_3OH + CH_2O \quad [11]$$

$$CH_3O + CH_3 \rightarrow CH_3OCH_3 \quad [12]$$

$$CH_3O + CH_3 \rightarrow CH_2O + CH_4 \quad [13]$$

$$CH_3O + CH_3CO \rightarrow CH_3OH + CH_2{=}CO \quad [14]$$

$$CH_3O + CH_3CO \rightarrow CH_2O + CH_3CHO \quad [15]$$

$$CH_3COCH_3 + Hg^* \rightarrow CH_3CO + CH_3 + Hg \quad [16]$$

$$CH_3OCH_3 + Hg^* \rightarrow CH_3O + CH_3 + Hg \quad [17]$$

$$CH_3OCH_3 + Hg^* \rightarrow CH_3OCH_2 + H + Hg \quad [18]$$

$$CH_3OCH_2 + OCH_3 \rightarrow CH_3OCH_2OCH_3, \quad [19]$$

and (*b*) from reactions [2]–[4]:

$$2CH_3 \rightarrow C_2H_6 \quad [20]$$

$$CH_3\overset{\overset{O}{\|}}{C}O \rightarrow CH_3 + CO_2 \quad [21]$$

$$\overset{\overset{O}{\|}}{C}OCH_3 \rightarrow CH_3 + CO_2 \quad [22]$$

$$\overset{\overset{O}{\|}}{C}OCH_3 + CH_3O \text{ (or } CH_3CO) \rightarrow H\overset{\overset{O}{\|}}{C}OCH_3 + CH_2O \text{ (or } CH_2{=}CO). \quad [23]$$

Since many of these products can be formed in several ways it is not possible to establish the relative importance of these secondary reactions. The reactions of radicals such as CH_3CO and CH_3O with a second excited mercury atom under conditions of intense illumination in the present apparatus cannot be entirely neglected (7). The reaction of secondary products such as CH_3OCH_3 with excited mercury must also occur. For example, $CH_3OCH_2OCH_3$ is most probably formed by the sequence of reactions [12], [18], and [19].

The relative importance of the primary step [1] can be estimated from the amount of products derived from CH_2O and from CH_3CO radicals according to the following relations:

$$R_{CH_3O} = 2R_{(CH_3O)_2CH_2} + R_{CH_3OCH_3} + R_{CH_2O} + R_{CH_3OH} \quad [24]$$

$$R_{CH_3CO} = 2R_{(CH_3CO)_2} + R_{CH_3CHO} + R_{CH_2=CO} + R_{CH_3COCH_3}. \quad [25]$$

The yield of CO cannot be included in either of these relations, since CH_2O and CH_3CO radicals may both give CO by reaction with Hg*. As shown in Table I, the yield of CH_3O amounts to 50–53% of the methyl acetate decomposed. The yield of CH_3CO is, however, only 34–38%. The difference presumably results from CH_3CO radicals which have disappeared to form products other than those listed in equation [25], that is, by reaction [7]. Since CH_3CO radicals would be thermally stable at 55° C, it is possible that some of the acetyl radicals carry over from the primary step sufficient energy to cause them to dissociate. Alternatively, reaction [7], like reaction [8], may be brought about by collision with an excited Hg atom.

An attempt was made to obtain an upper limit to acetyl production by introducing CH_3 radicals into the reaction stream and measuring the limiting yield of CH_3COCD_3. In this experiment, $Hg(CD_3)_2$ was added to the carrier gas as described in earlier publications (9, 10). The mercury-photosensitized decomposition of the $Hg(CD_3)_2$ then provided a large concentration of CD_3 radicals. According to Wijnen (2), the ratio of disproportionation to combination for $CH_3 + CH_3CO$ is about 0.06, and for the present purposes reaction [10] can be neglected compared to reaction [9]. The limiting yield of CH_3COCD_3 formed in the presence of a large excess of CD_3 should then provide an indication of the number of CH_3CO radicals which survive the primary step long enough to react with CD_3 radicals. The yields of CH_3COCD_3 obtained in this way are given in Table II. A plot of CH_3COCD_3 against added $Hg(CD_3)_2$ is given in Fig. 1. Although the yield is

TABLE II

CH_3COCD_3 formed by addition of CD_3 radicals to methyl acetate decomposition at 55° C
(Helium pressure 8 mm; methyl acetate partial pressure 10.34;
$Hg(CD_3)_2$ partial pressure in arbitrary units)

$Hg(CD_3)_2$ added	CH_3COOCH_3 decomposed (μ)	CH_3COCD_3 formed (μ)	CH_3COCD_3/CH_3COOCH_3 (decomp.)
15.0	1.24	0.115	0.093
29.0	1.21	0.172	0.142
42.5	1.24	0.237	0.191
59.5	1.24	0.287	0.231
93.0	1.22	0.322	0.264
113.0	1.22	0.344	0.282
140.5	1.10	0.347	0.315
192.0	1.08	0.364	0.337
273.0	0.96	0.357	0.372

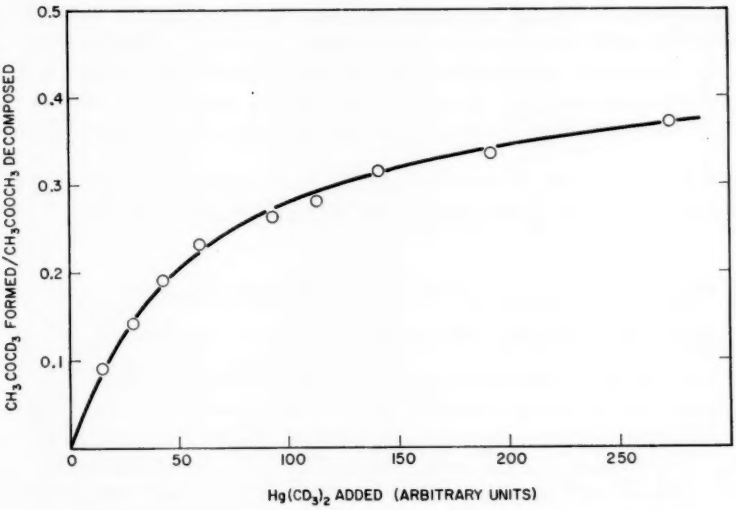

FIG. 1. Yield of CH_3CO radicals as shown by addition of CD_3 radicals to reaction stream.

still rising slowly even with the largest amount of added $Hg(CD_3)_2$, it is apparent that the limiting yield of CH_3CO is appreciably less than the 53% yield obtained for CH_3O. It is, in fact, not much higher than the CH_3CO yield in Table I as obtained from equation [28]. The difference between the values of 53% and of about 40% suggests that an appreciable fraction, possibly about one fifth, of the CH_3CO radicals formed in [1] decomposes to CH_3 and CO before the CH_3CO radicals have time to react with the other radicals present.

A slightly higher limit for the occurrence of reaction [1] can be obtained on the basis that

$$\text{total reaction [1]} = \tfrac{1}{2}(R_{CH_3O} + R_{CH_3CO} + R_{CO}). \qquad [26]$$

As shown in Table I, reaction [1] on this basis accounts for about 58% of the methyl acetate disappearing or about 82% of the total oxygen recovered.

The relative importance of the primary steps [2] and [4] can also be estimated from the yields in Table I. The only product other than CO_2 which could be formed only by reactions [2] and [4] was a small amount of methyl formate. In a separate experiment, a sample of reaction products was condensed from the gas stream in a liquid-nitrogen trap. G.L.P.C. fractionation of this sample yielded a fraction which by the retention time and by the mass spectrum was shown to be methyl formate and not the isomeric acetic acid. This points to the presence of $COOCH_3$ radicals resulting from reaction [2]. Confirmation of the presence of this radical was provided by the addition of CD_3 radicals to the reaction stream by means of $Hg(CD_3)_2$. The formation of a small peak at mass 77 (CD_3COOCH_2) supports the view that methyl formate was formed from $COOCH_3$ radical by reaction [23] rather than by combination of HCO and CH_3O radicals. The $COOCH_3$ radical was found in earlier work (8) to be reasonably stable at 55° C. The CH_3CO_2 radical, on the other hand, if formed at all, must dissociate immediately. The

extent of the primary steps [2] and [4], then, can be estimated from the sum of the yields of CO_2 and methyl formate in Table I to be 13% of the methyl acetate decomposed. This is 18% of the total oxygen recovered.

A search was made for the CH_2COOCH_3 and CH_3COOCH_2 radicals which would result from removal of a hydrogen atom in a primary step. In the presence of a large excess of added CH_3 radicals no ethyl acetate or methyl propionate could be detected. The CH_2COOCH_3 radical, if present, should have been detected in this experiment, since it must have an appreciable lifetime at 55° C. Wijnen found $CH_3OCH_2COOCH_3$ from the combination of CH_2O and CH_2COOCH_3 to be a product of the photolysis even at 217° C (2). In this case the CH_2COOCH_3 radical was formed by a hydrogen-atom abstraction by CH_3 or CH_3O. The absence of these radicals in the present experiments, together with the low yield of H_2 mentioned above, indicates that the amount of decomposition proceeding by removal of a hydrogen atom was quite small.

In view of the 25% of methyl acetate which disappears to form unidentified products, any conclusions as to the ratios of various primary modes of dissociation are rather unreliable. It appears, however, that of that fraction which does give identifiable products, about 82% decomposes by reaction [1] and the remainder by reactions [2] and [4]. The ratio $R_1/(R_2+R_4)$ is about 4.4:1. This is not greatly different from the ratio observed by Wijnen (2) in the direct photolysis using a S-500 Hanovia arc. Using the data in Wijnen's Table 1 (excepting runs No. 13, 14, and 20 for which the CO_2 yield is anomalously low), one obtains values for the ratio of CH_3CO production to CO_2 production ($2R_{(CH_3CO)_2}+R_{CH_3COCH_3}+R_{CO}/R_{CO_2}$) ranging from 3.6 to 5.1. Ausloos (5) also found that CO production predominated in the photolysis of esters with unfiltered light, but that the formation of CO_2 became equally important at longer wavelengths.

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FURTHER EVIDENCE FOR CYCLIC MONOMERS IN HEATED LINSEED OIL[1]

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ABSTRACT

The distillable esters of heated linseed oil contain a fraction, designated NAFD, which fails to form an adduct with urea. NAFD itself has now been separated into three fractions by gas–liquid partition chromatography. The chemical structure of these component esters was investigated by means of their infrared spectra and by analysis of the products obtained on oxidation of the esters with periodate–permanganate. It is concluded that all the components have the same carbon skeleton, namely, 1-propyl-2-alkenecarboxycyclohexene, but differ in the position of the double bond in the side chain.

INTRODUCTION

Wells and Common (1) have shown that the distillable (monomeric) ethyl esters obtained by ethanolysis of heated linseed oil contain a fraction, designated NAFD, that fails to form an adduct with urea. These authors suggested that NAFD contains a non-terminal ring structure preventing urea-adduct formation. Subsequently, MacDonald (2) obtained evidence for the presence of a six-membered non-terminal ring structure when he isolated phthalic anhydride following aromatization and oxidation of NAFD. MacDonald (2) also established that NAFD consisted of ethyl esters of C_{18} monocarboxylic acids containing an average of two double bonds per molecule. The double bonds did not contribute to the failure of NAFD to form a urea adduct because hydrogenated NAFD also failed to form an adduct (2). However, the low yield (about 1% of theory) of phthalic anhydride obtained (2) indicated that further investigation was necessary in order to determine the positions of the ring and the double bonds in the NAFD carbon chain.

In the present work, oxidation of NAFD by the periodate–permanganate oxidative procedure of Lemieux and von Rudloff (3) yielded a complex mixture of products, suggesting that NAFD was itself a mixture of C_{18} esters differing in the positions of the double bonds, of the ring, or both. On subjecting the methyl esters of NAFD acids to gas–liquid partition chromatography (G.L.P.C.), at least three components were detected. Two of the components were collected from the effluent gas stream and their structures were investigated separately by the periodate–permanganate oxidative procedure (3).

EXPERIMENTAL

Preparation of NAFD

NAFD was prepared from two distillation fractions of ethyl esters of heated linseed oil collected at 60° and 70°, respectively (2). The oxidation experiments were carried out with NAFD prepared from the fraction collected at 60° (NAFD 60°). Since this material was used up in these experiments, subsequent studies, e.g., fractionation of NAFD by G.L.P.C., were carried out with NAFD prepared from the fraction collected at 70° (NAFD 70°). The use of NAFD 70° was justified by the fact that (*a*) its refractive index (n_D^{20}, 1.47200) differed only slightly from the value (n_D^{20}, 1.47195) reported for NADF

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[3]*Deceased March 1, 1957.*

60° (2); (*b*) both preparations contained the same component esters (Fig. 1, Table I); and (*c*) their infrared spectra were identical (Fig. 2).

Oxidation of NAFD

Total NAFD (ethyl esters) or NAFD fractions (methyl esters) obtained by G.L.P.C. were converted to free acids by saponification with ethanolic 0.5 *N* potassium hydroxide, followed by removal of the ethanol *in vacuo*, acidification, and extraction with ethyl ether. The free acids were dissolved in sodium bicarbonate solution at pH 7.6 (prior conversion to soaps by titration with 1 *N* sodium hydroxide hastened solution of the acids) and were oxidized with periodate–permanganate according to the procedure of Lemieux and von Rudloff (3). In a typical experiment, 1.03 g (3.69 mmoles) of NAFD acids consumed 17.0 mmoles of periodate (115% of theory for oxidation of two double bonds) after 30 hours, and yielded 1.22 g (theory 1.50 g) of oxidation products containing 3.12 equivalents (theory 5.0 equivalents) of acid per mole of NAFD oxidized.

A portion of the oxidation products of NAFD 60° was methylated with diazomethane in ethyl ether, and the resulting methyl esters were partially fractionated by distillation at 0.05 mm Hg; 75% of the products was collected up to 134° and this fraction is designated as "volatile" products; the remaining material is designated as "non-volatile" products.

Analysis of Oxidation Products

(a) Gas–Liquid Partition Chromatography (G.L.P.C.)

G.L.P.C. was carried out by the method of James and Martin (4) using a Podbielniak "Chromacon" (series 947-3V) which was modified to take glass chromatographic columns and to improve the pressure regulator, temperature control, and injection and collection systems. Helium was used as carrier gas, and a katharometer was used as detector.

Analyses of the methyl esters of oxidation products were made at 197° on a 4-ft column of Apiezon M – Celite 545 (1:4, w/w) (5). Separation of the methyl esters of NAFD was carried out at 240° on an 8-ft column of the same stationary phase.

(b) Paper Chromatography

Oxidation products were chromatographed as free acids by the method of Kolbe (6) for the separation of dicarboxylic acids, on Schleicher and Schuell 2043B paper using ethanol – 6 *N* ammonia – 3 *N* ammonium carbonate (6:1:1, v/v) as solvent. The chromatograms were developed for 20 hours at room temperature, dried, and sprayed with a solution of methyl red in borate buffer (6) to detect the acids. To isolate the separated acids, the mixture was applied as a band to sheets of Schleicher and Schuell paper (ca. 43 mg/sheet) and chromatographed as described above. The separated components were located on a guide strip cut from the middle of the sheet, and were eluted from the remaining paper with 3 *N* ammonia solution. The acids were isolated by acidifying the concentrated eluates with 0.1 *N* HCl and extracting with ethyl ether.

It is important to note that paper chromatography will analyze the total oxidation products, whereas G.L.P.C. will analyze only the volatile products.

RESULTS

Fractionation of NAFD by G.L.P.C.

The methyl esters of NAFD 60° or NAFD 70° were resolved by G.L.P.C. at 240° into three fractions (Fig. 1), the concentrations and relative retentions of which are given in Table I. The three fractions from both preparations of NAFD had the same relative retentions but differed in their relative proportions. As might be expected, NAFD 70°

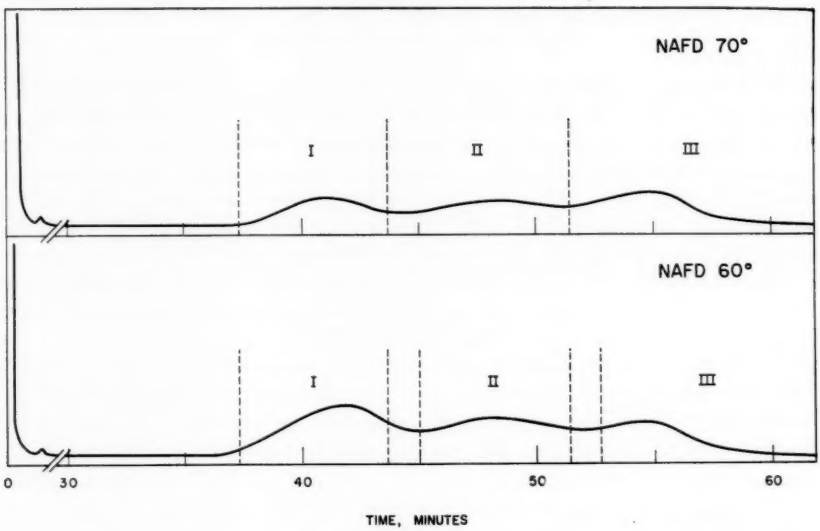


FIG. 1. Fractionation of NAFD 60° and NAFD 70° by G.L.P.C. on Apiezon M at 240°. Broken lines indicate collected fractions.

TABLE I

Components of NAFD methyl esters separated by G.L.P.C.

NAFD analyzed	Fraction	Relative retention*	Weight distribution, % of total
NAFD 60°	I	3.55	42
	II	4.05	31
	III	4.48	27
NAFD 70°	I	3.55	36
	II	4.05	25
	III	4.48	39

*Values are relative to that of methyl myristate on Apiezon M at 240°; relative retentions of standard methyl esters: palmitate, 2.21 and stearate, 4.67.

contained a higher proportion of fraction III and a lower proportion of fraction I than did NAFD 60°. Samples of each of the fractions (from NAFD 70°) were collected from the effluent gas stream for further investigation.

Examination of the infrared spectra of the fractions (Fig. 2) showed that the band at 660 cm^{-1}, considered characteristic of NAFD (2), was completely absent in the spectrum of fraction I, moderately intense in fraction II, and greatest in fraction III. Since fraction II appeared to be contaminated with both fractions I and III, only the latter fractions were subjected to further analysis. Anal. Calc. for methyl octadecadienate: iodine value, 173; C—CH_3, 9.20%. Found for fraction I: iodine value (7), 154; C—CH_3 (11), 9.13%. Found for fraction III: iodine value (7), 160; C—CH_3 (11), 8.20%.

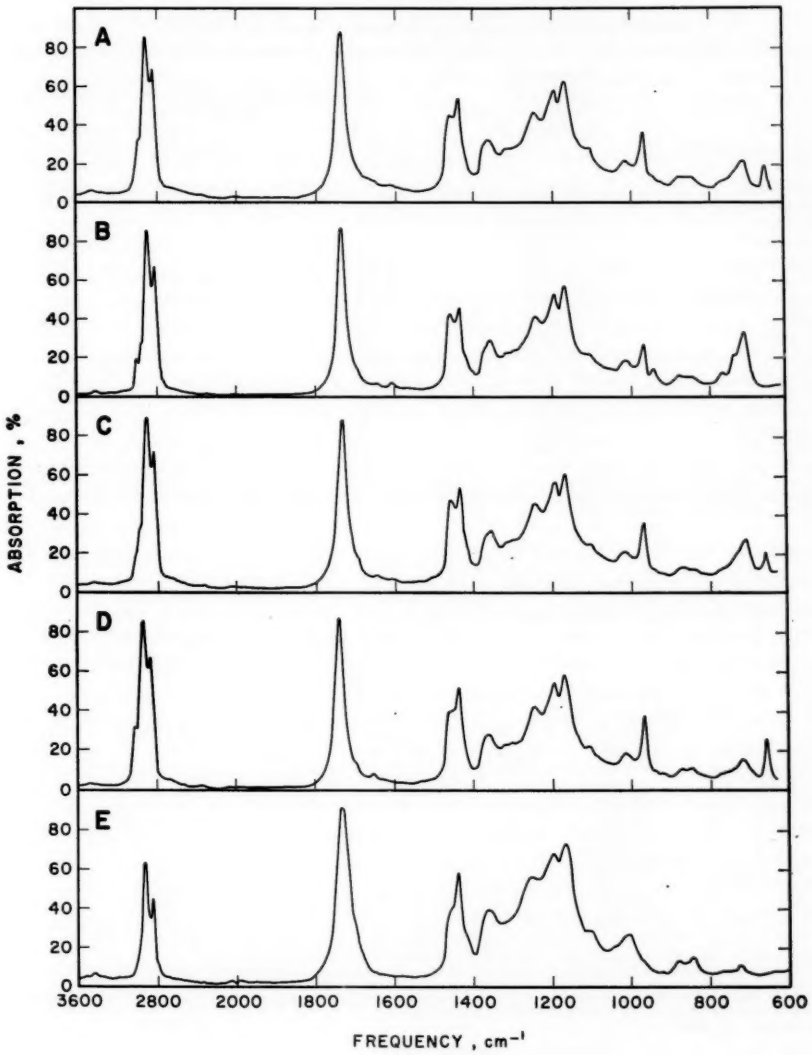


FIG. 2. Infrared spectra. A, NAFD 60° or 70°; B, fraction I; C, fraction II; D, fraction III; E, methyl ester of polycarboxylic acid from fraction III (purified by chromatography on silicic acid). Spectra were run on the undiluted oils.

Oxidation Products

Results of typical analyses of the oxidation products of total NAFD 60° and of fractions I and III by G.L.P.C. and by paper chromatography are presented in Tables II and III, respectively. NAFD 60° and fraction I yielded five major products, some of which

TABLE II

Analysis of oxidation products (as methyl esters) by G.L.P.C.

Identity of products	Retention relative to methyl myristate at 197°*	Relative molar concentration of oxidation products from:		
		NAFD 60°	Fraction I	Fraction III
Unidentified	0.0155	—	—	0.24
n-Hexanoate	0.0244	0.03	0.03	0.06
Succinate	0.0301	0.01	—	—
Unidentified	0.0320	—	—	0.04
n-Heptanoate	0.0378	0.01	—	—
Glutarate	0.0503	0.02	0.04	0.19
n-Propyl succinate	0.0746	0.75	0.65	—
Adipate	0.0820	Trace	—	0.53
Pimelate	0.131	0.13	0.10	0.37
Suberate	0.212	0.64	0.31	1.54
Unidentified	0.287	0.14	—	—
Azelate	0.342	1.00	1.00	1.00
Unidentified	0.455	0.21	—	—
Unidentified	0.506	0.44	—	0.80
Unidentified	0.532	—	0.31	—

*Values for standard methyl esters: valerate, 0.0142; *n*-hexanoate, 0.0245; succinate, 0.0292; *n*-heptanoate, 0.0377; glutarate, 0.0500; *n*-octanoate, 0.0623; *n*-propyl succinate, 0.0746; adipate, 0.0822; *n*-nonanoate, 0.098; pimelate, 0.132; *n*-decanoate, 0.156; suberate, 0.214; azelate, 0.344; sebacate, 0.562.

TABLE III

Analysis of oxidation products (as free acids) by paper chromatography*

Identity of acids	R_F value relative to sebacic acid‡	Relative concentration of acids from:†				
		NAFD 60°				
		Total products	"Non-volatile" products	"Volatile" products	Fraction I	Fraction III
Unidentified§	0.204	+	+	−	+	+
Unidentified§	0.298	+++	+++	−	−	++++
Unidentified	0.560	+	−	+	+	−
Adipic	0.611	−	−	−	−	++
Pimelic	0.721	−	−	−	−	+
n-Propyl-succinic	0.755	++	−	++	++	−
Suberic	0.820	++	−	++	++	+++
Azelaic	0.915	+++	−	+++	+++	++

*The system used (6) does not separate monocarboxylic acids, which move with solvent front.
†Relative concentration indicated by number of +'s; − indicates component not detected; only major components are revealed by the spray reagent (6).
‡Relative R_F of standard acids: oxalic, 0.095; malonic, 0.211; succinic, 0.424; glutaric, 0.516; adipic, 0.611; pimelic, 0.721; *n*-propyl-succinic, 0.755; suberic, 0.820; azelaic, 0.915; sebacic, 1.00.
§Probably tricarboxylic acids.

were identified as azelaic, *n*-propyl-succinic, suberic, and pimelic acids (in decreasing concentration). The products from fraction III consisted mostly of a tricarboxylic acid together with small amounts of dicarboxylic acids, some of which were identified as suberic, azelaic, adipic, pimelic, and glutaric acids (in decreasing concentration). The major dicarboxylic acid methyl esters from total NAFD 60°, after separation by G.L.P.C., were collected from the effluent gas stream, converted to free acids, and characterized by analysis and melting point.

n-Propyl-succinic acid.—This component, after recrystallization from benzene – petroleum ether (b.p. 40–60°), had m.p. 90–91°, alone or when admixed with authentic

n-propyl-succinic acid (m.p. 92–93°). Anal. Calc. for $C_7H_{12}O_4$: C, 52.49%; H, 7.55%; neutral equiv., 80.0. Found: C, 52.41%; H, 7.68%; neutral equiv., 79.8.

Suberic acid.—This component was recrystallized from water and had m.p. 139.5°, alone or when admixed with authentic suberic acid (m.p. 140–141°). Anal. Calc. for $C_8H_{14}O_4$: C, 55.16%; H, 8.10%; neutral equiv., 87.4. Found: C, 55.40%; H, 8.38%; neutral equiv., 87.4.

Azelaic acid.—This component, after recrystallization from benzene, had m.p. 104.5–105°, alone or in admixture with authentic azelaic acid (m.p. 106.5°). Anal. Calc. for $C_9H_{16}O_4$: C, 57.43%; H, 8.57%; neutral equiv., 94.0. Found: C, 57.67%; H, 8.42%; neutral equiv., 93.6.

The "volatile" fraction of the oxidation products of NAFD 60° on analysis by G.L.P.C. showed the same components as the total oxidation products of NAFD 60° given in Table II. The "non-volatile" products showed no peaks up to methyl sebacate under the same conditions, but on paper chromatography showed two components with R_F values (relative to sebacic acid) of 0.204 and 0.298 (Table III). These acids were almost completely absent from the oxidation products of fraction I, but were the predominating products from fraction III (Table III). Since these "non-volatile" products had low R_F values on paper chromatography, and had longer retention times than methyl sebacate on G.L.P.C., it was tentatively concluded that they were tricarboxylic acids of molecular weight greater than sebacic acid.

The tricarboxylic acid components were isolated by large-scale paper chromatography of 435 mg of the "non-volatile" fraction of total NAFD 60°; 32.9 mg of the acid with R_F 0.204 and 402.0 mg of the acid with R_F 0.298 were obtained as oily residues which failed to crystallize. Only the component with R_F 0.298 was investigated further. Its methyl ester showed strong ester absorption in the infrared at 1730 cm^{-1}, as well as a weak lactone band at 1775 cm^{-1}. Chromatography of the methyl ester on silicic acid with chloroform as eluant yielded a fraction (170 mg), the infrared spectrum of which showed only the ester band at 1730 cm^{-1}. The ester gave only one peak, with retention relative to methyl myristate of 3.09, when analyzed by G.L.P.C. on Apiezon M at 240°. The relative retention of the ester indicated that it had a molecular weight not less than that of methyl heptadecanoate (M.W., 284; relative retention, 3.20). Anal. Found: C, 61.55%; H, 8.89%; C—CH_3 (11), 7.20%; OCH_3, 24.1%; saponification equivalent, 117. Calc. for $C_{16}H_{19}(COOCH_3)_3$: C, 60.7%; H, 8.92%; C—CH_3, 8.55%; OCH_3, 29.4%; saponification equiv., 105.4. Calc. for $C_{11}H_{21}(COOCH_3)_3$: C, 61.8%; H, 9.14%; C—CH_3, 8.18%; OCH_3, 28.1%; saponification equiv. 110.0.

The analytical and chromatographic data suggest that the ester is a trimethyl ester of a C_{13} or C_{14} tricarboxylic acid with one terminal methyl group.

DISCUSSION

The analysis of NAFD by G.L.P.C. showed that it contained at least three components (Fig. 1, Table I), the relative retentions and analyses of which were consistent with those of methyl esters of diunsaturated C_{18} fatty acids (5). These esters cannot be simple open-chain diunsaturated compounds since it has already been shown that NAFD (1) and hydrogenated NAFD (2) fail to form an adduct with urea. The failure of NAFD to form a urea adduct might be due either to the presence of a non-terminal ring (1, 2) or to the presence of branching in the fatty-acid chain. However, the oxidation products of NAFD or of fractions I and III contained only traces of lower fatty acids (propionic through caproic, Table II) and failed to form oximes or hydrazones, indicative of the absence of

ketones. Furthermore, terminal methyl values for fractions I and III indicated the presence of only one C—CH_3 group per molecule. Since a branched-chain unsaturated fatty acid would yield either a ketone or a monocarboxylic acid on oxidation, and would have more than one terminal methyl group per molecule, it follows that none of the NAFD components can be branched, and therefore, that they contain a non-terminal ring.

Examination of the infrared spectra of the components of NAFD (fractions I, II, and III; see Fig. 2) revealed that the band at 660 cm^{-1}, attributed to the C—H out-of-plane deformation of a cis-disubstituted double bond in a six-membered ring (2), was present in III (and to a lesser extent in II) but not in I. Furthermore, the intensity of the band at 970 cm^{-1}, attributed to a trans-disubstituted double bond (8, 9), increased in the order I < II < III, and the weak absorption at 1650 cm^{-1}, due to the C=C double-bond stretching vibration (9), also increased in the same order. Finally the absorption at 715 cm^{-1}, characteristic of the methylene rocking vibration (9), decreased in intensity in the order I > II > III.

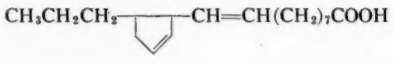
These data suggest that fraction III contains one trans-disubstituted double bond, one cis-disubstituted double bond (probably in a six-membered ring), and short methylene chains, whereas fraction I contains no trans-disubstituted double bonds and longer methylene chains. The weak absorption at 970 cm^{-1} (trans double bond) in fraction I is probably due to the presence of a small amount of fraction II.

Separation of the components of NAFD by G.L.P.C. on Apiezon M may be attributed largely to differences in stereochemistry at the unsaturated centers, since compounds with trans double bonds have longer retention times than the cis isomers (see James (10)). The operation of other factors, such as the position of the ring and the lengths of the methylene chains, may be responsible for the incomplete separation and overlapping of the components, as evidenced by the complexity of the mixtures of oxidation products obtained from fractions I and III (Tables II and III). Nevertheless, the data in Tables II and III show that the major oxidation products of fraction I are azelaic and *n*-propyl-succinic acids, together with smaller amounts of suberic, pimelic, and an unknown acid, and those of fraction III are the tricarboxylic acid, suberic acid, and azelaic acid, together with smaller amounts of adipic, pimelic, and an unidentified acid. On the basis of the formation of these major oxidation products, one can suggest possible structures which would at least account for a major part of the material in fractions I and III.

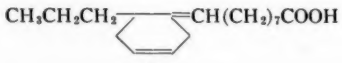
Thus the formation of *n*-propyl-succinic and azelaic acids from fraction I indicates that the latter contains the two fragments

$$CH_3CH_2CH_2—\underset{\displaystyle CH_2—C=}{\overset{\displaystyle |}{CH}}—C= \quad \text{and} \quad =CH(CH_2)_7COOH.$$

These fragments account for 16 carbon atoms and 3 double bonds, whereas fraction I contains 18 carbon atoms and only 2 double bonds. Formation of azelaic acid fixes the carboxyl end of the structure and also the position of one double bond, while the *n*-propyl-succinic acid fixes the methyl end of the structure. Two possible structures, A or B, could account for these products.

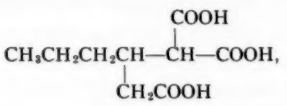


A

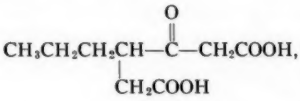


B

Structure A, on oxidation, would give rise to azelaic acid and an acid,

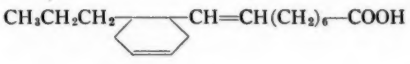


which would be oxidatively decarboxylated to *n*-propyl-succinic acid. Structure B would yield azelaic and a β-keto acid,

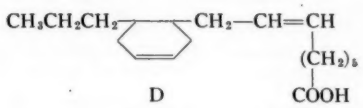


which would also be oxidatively decarboxylated to *n*-propyl-succinic acid. However, structure B fits the infrared data better than A, since B would not be expected to absorb strongly at 970 cm^{-1} (characteristic of a trans-disubstituted double bond) whereas the double bond in the side chain of A would be in the more thermodynamically stable trans conformation, as a result of the conditions of formation (2), and would thus absorb strongly at 970 cm^{-1}.

Formation of suberic acid and pimelic acid from fraction I might be accounted for by the presence of compounds (C and D) with a modified structure B, in which the exocyclic double bond is displaced by one or two carbon atoms towards the carboxyl group.



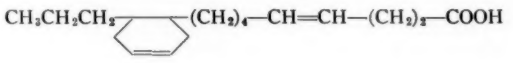
C



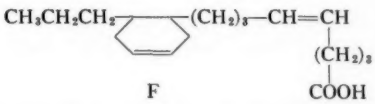
D

Oxidation of C and D would yield, in addition to suberic and pimelic acids, C_{10} and C_{11} tricarboxylic acids, respectively, possibly accounting for the small amount of tricarboxylic acids detected on paper chromatograms of the oxidation products of fraction I (Table III). It is quite likely that the bulk of fraction II consists of these two acids (C and D), incompletely resolved from the component with structure B present in fraction I.

To account for the formation of large amounts of C_{13} or C_{14} tricarboxylic acid from fraction III, structures E and F are postulated.



E



F

Oxidation of structures E and F would yield a C_{14} tricarboxylic acid and succinic acid, and a C_{13} tricarboxylic acid and glutaric acid, respectively. The presence of glutaric acid in the oxidation products of fraction III (Table II) adds additional support for structure F. Suberic and pimelic acids in the oxidation products of fraction III probably arise from structures C and D, which were not completely separated from fraction III.

The proposed structures should account for the fact that fraction I does not absorb at 660 cm^{-1}, the region attributed to C—H out-of-plane deformation of a cis double bond in a six-membered ring. All the structures proposed (B, C, D, E, and F) contain a cis double bond in a six-membered ring, but in structure B (the major component of

fraction I), the second double bond is attached directly to the ring. It is possible that this double bond hinders the C—H out-of-plane deformation of the ring double bond to the extent that no absorption at 660 cm^{-1} occurs, whereas the exocyclic double bonds in structures C, D, E, and F, being further removed, offer no such hindrance.

Finally, the proposed structures should be consistent with the finding (2) that NAFD gave low yields of phthalic anhydride after allylic bromination, dehydrohalogenation, and oxidation of the side chains. The steric requirement for dehydrohalogenation in a six-membered ring is that the halogen and hydrogen atoms must be trans-diaxially oriented in the transition state (12). This requirement could not easily be fulfilled by an unsaturated six-membered ring such as in the proposed structures (particularly structure B), and therefore low yields of phthalic anhydride would be expected from any of these structures.

ACKNOWLEDGMENTS

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THE DIFFERENTIAL THERMAL ANALYSIS OF WOOD[1]

D. F. ARSENEAU

ABSTRACT

Differential thermal analysis was used to study the thermal breakdown of balsam fir and its components in air from 50° to 420° C. In this range, the thermogram of balsam fir is simply a composite of the individual thermograms of the components of the wood, with little, if any, interaction between the components.

INTRODUCTION

Wood is a multicomponent, molecularly non-homogeneous material in which part of the components exist in continuous separate intermingling structures. Balsam fir is reported (1) to contain about 27.7% lignin, 49.4% alpha-cellulose, 15.4% hemicelluloses, 1.5% acetyl, 0.5% ash, and 4.6% extractives. These extractives comprise an extraordinary diversity of substances and could include fats, fatty acids, resin acids, resins, waxes, tannins, phlobaphenes, coloring matters, gums, starches, and many others. All of these components contribute certain physical and chemical properties to the wood substance. Differential thermal analysis (D.T.A.) is able to determine to some degree what contributions some of these components make to the thermal decomposition characteristics of the wood.

Previous workers have used D.T.A. to study lignin (2), cellulose (3), and the carbonation of wood (2, 4). One study (2) indicated that the pyrolysis of birchwood was accompanied by considerable interaction between its components. Since preliminary work on balsam fir did not indicate this same interaction, a further study was undertaken.

EXPERIMENTAL

Apparatus

The apparatus for this study consisted of a vertical-type brass furnace (Fig. 1) in which three identical holes were drilled to a depth of $2\frac{3}{4}$ inches. Great care was taken to see that these were equidistant from the perimeter of the furnace. Two of these holes were to receive the sample holders, the third was to receive the thermocouple that feeds a signal to the temperature controller. The furnace was wound with 20 ft of 24 gauge Chromel 'A' wire, wrapped in asbestos, and placed in a metal container 5 in. in diameter. Glass wool between the furnace and the container provided further heat insulation. The sample holders were brass tubes fitted with iron–constantan thermocouples and had a capacity of 0.7 ml, although their total capacity was not always utilized. These holders were inserted vertically through the bottom of the furnace.

The temperature of the furnace was regulated using an F and M Model 40 Linear Temperature Programmer with an iron–constantan thermocouple. A heating rate of 5.8° C per min was used in all determinations in this study. The signal from the differential thermocouples was preamplified by a Leeds and Northrup Model 9835-B Stabilized D-C Microvolt Indicating Amplifier and was recorded with a Varian Graphic Recorder Model G-10 with a span of 10 mv. Stable, reproducible results were obtained using this arrangement.

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Contribution from the Department of Chemistry, Xavier Junior College, Sydney, Nova Scotia.

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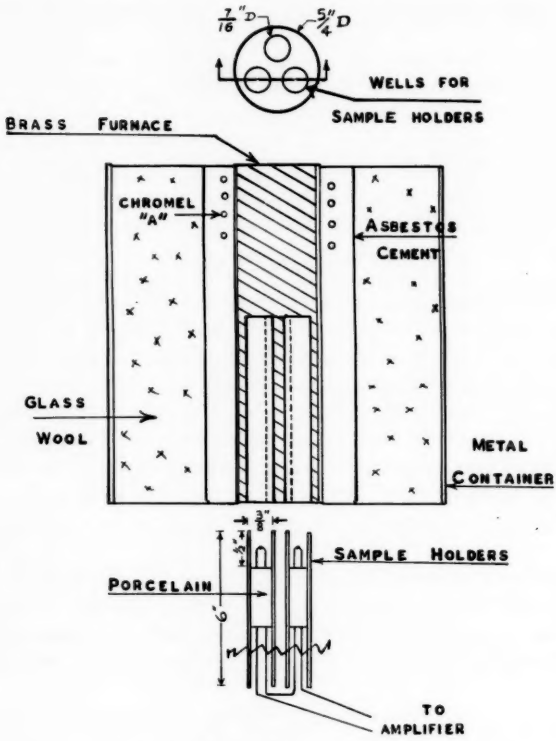

FIG. 1. Essentials of furnace and sample holders.

Wood Preparation

Air-dry balsam fir (*Abies balsamea* (L.) Mill.) was powdered using a metal file and then screened. That fraction passing mesh No. 70 but retained by mesh No. 200 was used in this work.

Removal of Extractives

Wood powder was extracted for 8 hours at room temperature with a mixture of 65 parts benzene, 35 parts ethanol, and 15 parts water. This mixture resulted in two layers. Upon evaporation, the benzene–alcohol layer yielded a brown, tarry material which was believed to contain resins. The alcohol–water layer was allowed to evaporate to near dryness at room temperature and was then further dried at 110° C. It yielded a gray material which was easily powdered. Some of the mixture appeared crystalline, and was very hygroscopic. A water solution of this material, acidified with acetic acid, gave a white precipitate with a solution of lead acetate, indicating the presence of tannins.

After the benzene–alcohol–water extraction, the wood powder was dried, and further extracted with 65 parts benzene and 35 parts ethanol. No extract was obtained. The wood was washed with water at 60° C and vacuum-dried at 100° C for 48 hours. This wood was designated as "free of extractives".

Isolation of Lignin

Two grams of wood free of extractives was added to 25 ml of 72% sulphuric acid at 10° C and stirred for 1 minute. The temperature was allowed to rise to 21° C. This mixture was allowed to stand for 2 hours with frequent stirring. The mixture was then diluted to 3% acid concentration, and boiled for 4 hours. The lignin residue was then filtered off using a fritted-glass crucible and washed with a liter of hot water until acid free. The lignin was dried at 110° C under vacuum for 24 hours.

Isolation of Some Hemicelluloses

One gram of wood powder free of extractives was treated with 50 ml of 20% potassium hydroxide for 18 hours under an inert atmosphere. Sufficient water was added to reduce the concentration of the potassium hydroxide to 3%, and the mixture was allowed to stand at 65 to 70° C for 2 hours before filtration. Excess acetic acid was added to neutralize the potassium hydroxide, and the hemicelluloses precipitated, twice the volume of methanol being used. After settling for about 2 hours, the precipitate was filtered off using a fritted-glass crucible, washed free of acid, washed with acetone, and vacuum-dried in a desiccator at room temperature.

Preparation for an Analysis

Calcined alumina, kept at 400° C until needed, was placed in both holders, the inert-sample holder being completely filled, the active-sample holder being filled to about $\frac{1}{8}$ of an inch of the thermocouple junction. Five milligrams of the active sample mixed with an equal volume of alumina was packed around the junction. Alumina was used to complete the filling of the sample holder. The two sample holders were inserted vertically into the furnace and a very slow current of air was circulated around the sample.

RESULTS AND DISCUSSION

Figure 2 shows eight thermograms over a range of 50° to 420° C. The interesting portions of the thermogram for air-dry wood powder have been lettered. Although some are very minor endotherms or exotherms, they reoccurred in all of the thermal analysis runs on the air-dry wood. As shown in Table I, all of the inflections except C can

TABLE I

Summary of peaks and their causes

Peak from air-dry wood	Fraction
A, endotherm at 145° C	Alcohol–water extract
B, endotherm at 163° C	Alcohol–water extract
C, 210° C	Unaccounted
D, 265° C	Possibly acid lignin
E, exotherm at 285° C	Benzene–alcohol extract
F, exotherm at 300° C	Sum of benzene–alcohol extract and acid lignin
G, exotherm at 330° C	Cellulose
H, exotherm at 360° C	Same as F

be attributed to reactions in the various fractions that go to make up the wood substance.

Thermogram II of the alcohol–water extract of wood discloses two endotherms at 144° and 163° C which could account for endotherms A and B. The extract melts at the former temperature and decomposes into a gas and a white powder at the latter.

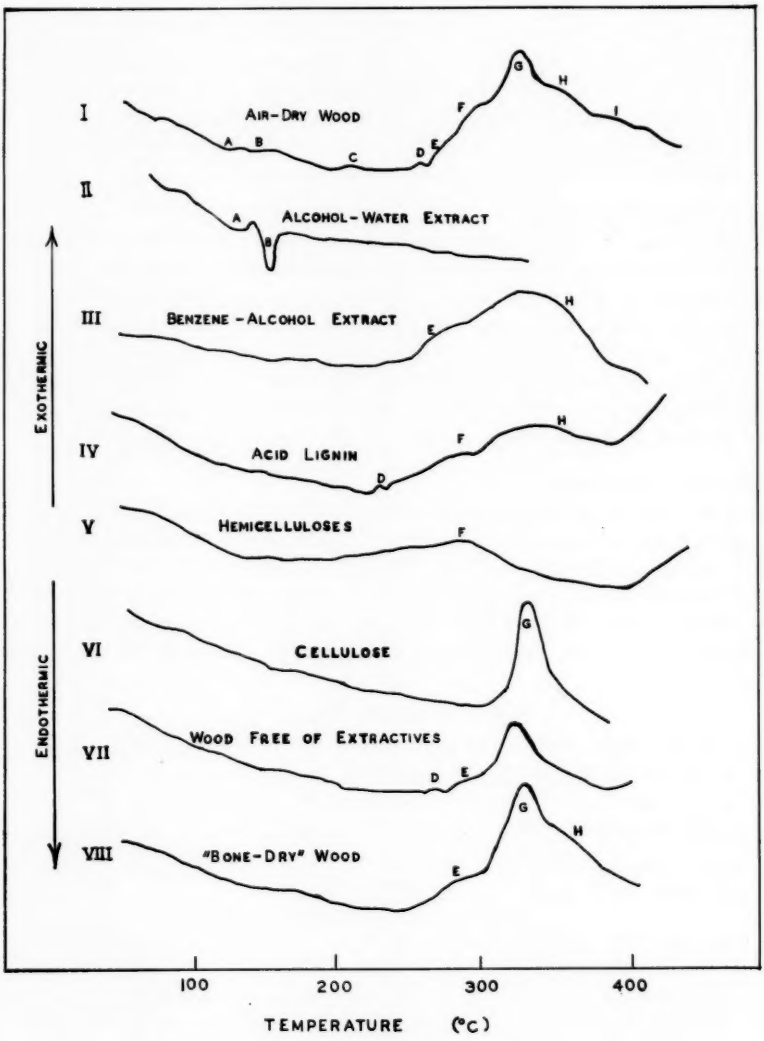

FIG. 2. Thermograms of balsam fir and its components.

The isolated acid lignin (IV) shows activity around 230° C quite similar to the variations D at 265° C in the thermogram for wood. This displacement could result from physical or chemical changes occurring in the lignin during the isolation procedure.

The main system of exothermic peaks from 270° to 400° C is actually a composite of several exothermic reactions. The shoulder E can be traced to the first portion of the main exothermic reaction of the benzene–alcohol extract (III). Section F is the sum of the tailing portion of the foregoing exotherm plus the 290° C exotherm of the acid lignin (IV).

Peak G is quite clearly the cellulose exotherm (VI) superimposed on the less intense exotherms of the lignin and the benzene–alcohol extract.

The hemicelluloses isolated by the procedure outlined above gave an exothermic peak which gradually reached a maximum at 285° C.

Morita and Rice (3) report that cellulose is characterized by an endotherm at 340° C when heated in an atmosphere of nitrogen. This present work shows that in air the endotherm becomes an exotherm at 330° C. The minor temperature shift can be attributed to the slower heating rate used here.

It is interesting to compare the thermograms of acid lignin and of the benzene–alcohol extract. Their marked similarity would suggest the presence of native lignin in the extract. In fact, a small amount (8 to 10%) of the protolignin in wood can be extracted in an unchanged form using indifferent solvents. Such lignin is termed "native" lignin. An acetone extract of wood, which would contain mainly resins, gave a thermogram identical with that of the benzene–alcohol extract. This similarity between the thermograms of lignin and resins could be expected because of a similarity in their basic structures.

These two thermograms do differ above 400° C, however. The acid lignin indicates the beginning of an intense exotherm, while the benzene–alcohol extract does not, nor does the unextracted wood powder show an exotherm in this range. Obviously the lignin has undergone some changes upon its isolation.

For thermogram VIII the original wood powder was vacuum-dried over drierite at 80° C for 48 hours. It was then left over drierite for 3 months at room temperature. Five milligrams of it was mixed with an equal volume of alumina and thermogramed. Many of the previously mentioned peaks were missing or decreased in intensity. It is difficult to account for these differences unless adsorbed water assists the breakdown of wood in a hydrolytic manner.

As would be expected, the thermogram of wood powder free of extractives (VII) displays the characteristic exotherm of cellulose at 330° C, and to a lesser extent, the exotherm of lignin, but not the peaks characteristic of the extractives.

It would appear, therefore, that over the range from 50° to 420° C the differential thermogram for balsam fir when heated in air is simply a composite of the individual thermograms of the various components of the wood, with little, if any, interaction between these components.

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THE REACTION BETWEEN METHYL RADICALS AND ISOBUTANE[1]

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ABSTRACT

It has been confirmed that the apparent rate constant for the reaction between methyl radicals, produced by the photolysis of deuterated acetone, and isobutane increases with decreasing isobutane pressure. An explanation is proposed to account for this observation suggesting that the production of methane by disproportionation between methyl and *t*-butyl radicals was not negligible as has been assumed previously.

INTRODUCTION

Observations made recently in this laboratory indicated that values of the apparent rate constants for the abstraction of hydrogen by methyl radicals, calculated using conventional methods, depended on the pressure of the added hydrogen donor. The reaction of methyl radicals with isobutane using the pyrolysis of di-*t*-butyl peroxide as a source of methyl radicals is an example of such a system (1). When mixtures of deuterated acetone and hydrogen were photolyzed, the rate constant for the reaction of methyl radicals with hydrogen was found to be a function of the hydrogen pressure (2). In view of the apparent similarities between the two systems, an investigation of the photolysis of deuterated acetone – isobutane mixtures was made.

EXPERIMENTAL

The apparatus and methods of analysis were similar to those employed in the photolysis of the deuterated acetone – hydrogen mixtures (2, 3). The deuterated acetone consisted of 98.2% acetone-d_6 and 1.8% acetone-d_5. Phillips Research Grade isobutane was outgassed by bulb-to-bulb distillations at $-160°$ C. The temperature of the reaction cell was $471 \pm 0.5°$ K for all experiments.

RESULTS AND DISCUSSION

In the photolysis of mixtures of deuterated acetone and isobutane, it is usually assumed that methane and ethane are formed by the following reactions:

$$CD_3 + CD_3 \rightarrow C_2D_6 \qquad [a]$$

$$CD_3 + CD_3COCD_3 \rightarrow CD_4 + CD_2COCD_3 \qquad [b]$$

$$CD_3 + i\text{-}C_4H_{10} \rightarrow CD_3H + t\text{-}C_4H_9. \qquad [c]$$

When the deuterated acetone was photolyzed alone (2), small amounts of CD_3H were formed due to the presence of acetone-d_5 in the sample, and its yield was given by

$$R_{CD_3H}/R_{C_2D_6}^{1/2} = 0.014[A] \times 10^{-12} \text{ (molecules/cc sec)}^{1/2}, \qquad [1]$$

where $[A]$ is the acetone concentration in molecules/cc. Allowance for this CD_3H was made when calculating values of $k_c/k_a^{1/2}$.

$$\rho = (k_c/k_a^{1/2})_{app} = R_{CD_3H}/R_{C_2D_6}^{1/2}[i\text{-}C_4H_{10}] \qquad [2]$$

Values of ρ are shown in Table I and it is evident that the apparent rate constant

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TABLE I

Products of the reaction at 471° K

Run	Time, sec	Concn., 10^{18} molecules/cc			Rate, 10^{12} molecules/cc sec					Ratio of rate constants, 10^{-13} (molecules/ml)$^{-1/2}$ sec$^{-1/2}$	
		d-Acetone	*i*-C_4H_{10}	*n*-C_4F_{10}	CO	Methane	Ethane	% CD_4	% C_2D_6	$k_b/k_a^{1/2}$	ρ
163	3,600	2.17	0.118		6.01	4.06	2.92	62.3	98.2	6.88	74.0
162	3,600	2.15	0.324		7.63	6.61	2.96	39.1	98.1	7.05	72.2
172	3,600	2.15	0.328		5.80	5.36	2.06	38.9	98.2	6.79	69.5
168	3,600	2.15	0.311	3.24	5.91	5.23	2.13	39.7	98.1	6.66	67.8
178	4,500	2.16	0.299	3.29	5.99	5.25	2.13	39.7	98.2	6.64	69.4
161	7,200	2.17	0.645		7.60	8.44	1.94	25.3	97.9	7.12	70.6
176	7,260	2.16	0.676		5.41	6.51	1.14	24.3	98.0	6.90	68.3
160	5,400	2.17	1.08		7.54	9.92	1.30	17.0	98.0	6.91	67.3
164	9,000	2.17	2.19		7.04	11.1	0.525	9.64	97.7	6.89	63.7
166	18,000	0.652	1.09		2.22	3.39	0.204	5.91	97.6	6.88	65.5
165	10,800	1.09	1.08		3.50	5.06	0.434	9.63	97.8	6.85	64.7
171	10,830	1.09	1.07	1.06	3.22	4.80	0.390	9.55	97.8	6.81	65.2
169	7,241	1.09	1.08	3.22	3.76	5.30	0.495	9.60	97.7	6.73	63.2
173	6,300	3.23	1.07		7.52	10.1	1.22	23.3	98.1	6.69	65.3

determined by equation [2] varies with the isobutane pressure, which confirms the earlier observation (1). Unlike the addition of hydrogen (2), however, the addition of isobutane to acetone-d_6 did not affect the ratio $R_{CD_4}/R^{1/2}_{C_2D_6}[A]$ in any way. Finally, ρ did not appear to be influenced by the addition of perfluoro-*n*-butane which indicates the absence of excited species in the system.

To account for these observations, it is proposed that the disproportionation reaction between methyl and *t*-butyl radicals was important in the systems discussed here, and that the observed variation of ρ with isobutane pressure is due to the assumption that this reaction was negligible. By inclusion of the following radical–radical reactions in the mechanism, allowance for this additional source of CD_3H can be made in the following manner:

$$CD_3 + t\text{-}C_4H_9 \rightarrow CD_3H + i\text{-}C_4H_8 \quad [d]$$

$$\rightarrow CD_3C_4H_9 \quad [e]$$

$$t\text{-}C_4H_9 + t\text{-}C_4H_9 \rightarrow i\text{-}C_4H_{10} + i\text{-}C_4H_8 \quad [f]$$

$$\rightarrow C_8H_{18} \quad [g]$$

$$CD_3 + CD_2COCD_3 \rightarrow C_2D_5COCD_3 \quad [h]$$

$$t\text{-}C_4H_9 + CD_2COCD_3 \rightarrow (CH_3)_3CCD_2COCD_3 \quad [i]$$

$$CD_2COCD_3 + CD_2COCD_3 \rightarrow CD_3COCD_2CD_2COCD_3. \quad [j]$$

The abstraction of deuterium from acetone-d_6 by t-C_4H_9 radicals is neglected on thermochemical grounds. On the basis of statistics, it is assumed that

$$2k_a = (2k_f + k_g) = 2k_j = k_d + k_e = k_h = k_i = k. \quad [3]$$

Although this expression is not entirely correct due to differences in masses and entropies of the collision complexes, the error will be small (see Appendix I).

By the use of [3] and the mass balances in CD_3, t-C_4H_9, and CD_3COCD_2 radicals, the following expressions for m were derived (see Appendix II):

$$m_{\mathrm{I}} = 1 - [\beta\lambda_E^{1/2}(1 - \lambda_D - \lambda_E^{1/2})/\lambda_H] \quad [4]$$

$$m_{\mathrm{II}} = ([1 + \beta(1 - \lambda_D)]/2\beta\lambda_H)\{1 - [1 - (4\beta\lambda_H)/(1 + \beta(1 - \lambda_D))^2]^{1/2}\} \quad [5]$$

$$m_{\mathrm{III}} = (1 - \lambda_D - \lambda_E^{1/2})/\lambda_H \quad [6]$$

$$m_{\mathrm{IV}} = 1/(1 + \beta\lambda_E^{1/2}), \quad [7]$$

where $\beta = k_d/(k_d + k_e)$, $\lambda_D = R_{CD_4}/2R_{CO}$, $\lambda_H = R_{CD_3H}/2R_{CO}$, $\lambda_E = R_{C_2D_6}/R_{CO}$, and m is the yield of CD_3H from [*c*] relative to the total yield of CD_3H (i.e., $m = k_c[CD_3][i\text{-}C_4H_{10}]/R_{CD_3H}$).

Values of m were calculated for $\beta = 0.446$ corresponding to $k_d/k_e = 0.806$. This was calculated from the data of Kerr and Trotman-Dickenson (4) using [3] rather than [3*a*] (Appendix I), which was employed by the original authors. This procedure is logically more consistent with the present work than the use, directly, of the value $k_d/k_e = 0.699$ as reported by the original authors. In the recalculation, k_f/k_g was taken as 4.44, being the average of the values reported by Kraus and Calvert (5). It is interesting to note that the recalculated value is in better agreement than the original with the less precisely but more directly determined value $k_d/k_e = 0.9$ found by McMillan (6) using a method which did not require the assumption of either [3] or [3*a*]. Frey's value (7) is also based on the assumption of [3*a*] but no data are given to permit recalculation.

It was assumed throughout that CH_3 and CD_3 behave identically in respect to their reactions with t-C_4H_9 radicals.

The agreement among the numerical values of m_{I}, m_{II}, m_{III}, and m_{IV}, Table II, while

TABLE II

Correction factors and corrected constants

Run	m_{I}	m_{II}	m_{III}	m_{IV}	λ_{E}	$10^{12}\,\rho m_{IV} = 10^{13} k_c/k_a^{1/2}$	β
163	0.752	0.737	0.805	0.765	0.477	56.8	0.353
162	0.773	0.783	0.828	0.785	0.378	56.8	0.338
172	0.782	0.788	0.831	0.792	0.347	54.9	0.346
168	0.770	0.786	0.867	0.790	0.355	54.5	0.257
178	0.764	0.781	0.893	0.791	0.352	57.1	0.203
161	0.806	0.808	0.871	0.818	0.250	57.5	0.294
176	0.821	0.824	0.879	0.831	0.208	56.9	0.304
160	0.839	0.838	0.877	0.845	0.169	56.8	0.342
164	0.889	0.884	0.920	0.893	0.0729	57.0	0.322
166	0.878	0.873	0.914	0.883	0.0894	57.9	0.316
165	0.861	0.857	0.896	0.866	0.120	56.2	0.337
171	0.867	0.869	0.872	0.867	0.118	56.9	0.428
169	0.856	0.852	0.901	0.862	0.129	54.8	0.306
173	0.845	0.843	0.863	0.848	0.162	55.5	0.393
					Mean	56.4	0.324
					Standard deviation	0.3	0.015

not surprising in view of the algebraic identity which can be shown to exist (Appendix II), is, nevertheless, very gratifying when the algebraic complexity of some of the expressions is considered. Values of $k_c/k_a^{1/2}$ were determined using m_{IV} and equation [8]; they are independent of the pressure of isobutane (Table II and Fig. 1).

$$k_c/k_a^{1/2} = \rho m_{IV} \qquad [8]$$

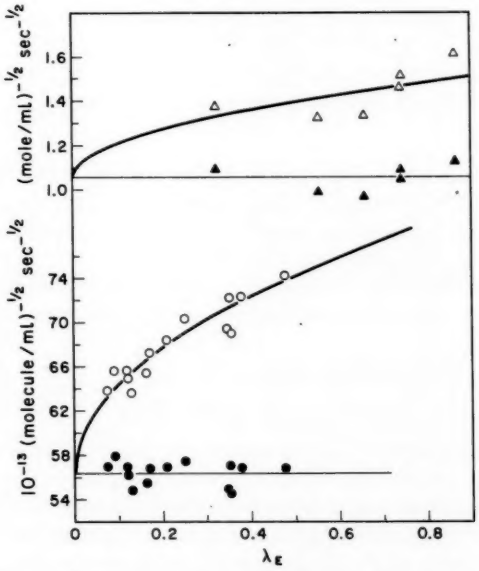

FIG. 1. Variation of apparent and corrected rate constants. *d*-Acetone, *i*-C_4H_{10} system (471° K): ○ ρ; ● $k_c/k_a^{1/2}$. DTBP, *i*-C_4H_{10} system (411° K): △ $R_{CH_4}/R_{C_2D_6}^{1/2}[i\text{-}C_4H_{10}]$; ▲ $k_c/k_a^{1/2}$. In both cases the curve is drawn from equation [8].

By equating any two of the expressions [4], [5], [6], and [7] it is found that β is given by the relation

$$\beta = [\lambda_H - (1-\lambda_D-\lambda_E^{1/2})]/\lambda_E^{1/2}(1-\lambda_D-\lambda_E^{1/2}). \quad [9]$$

Because of the many subtractions involved, measurements of β made in this way cannot be regarded as highly precise; this is shown by the considerable scatter of its values (Table II).

Inspection of equation [7] indicates that m decreases with increasing λ_E, i.e. with increasing light intensity or decreasing isobutane pressure, and tends to $1/(\beta+1)$ in the limit. In Fig. 2, m_{IV} is plotted against the logarithm of λ_E (equation [7]) and approaches

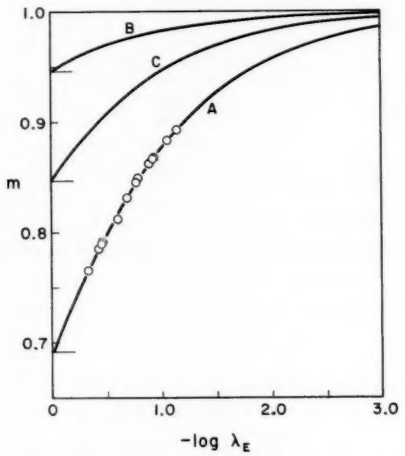

FIG. 2. Variation of m_{IV} with λ_E. A: m_{IV}, $\beta = 0.446$; B: m_{IV}, $\beta = 0.057$; C: m_{IV}, $\beta = 0.18$. The points on A are taken from Table II.

unity only when λ_E becomes exceedingly small; e.g. $m = 0.95$, 0.98, and 0.99 when $\lambda_E =$ 0.0139, 0.0021, and 0.0005 respectively. Consequently, to carry out experiments under conditions where the disproportionation reaction [*d*] would be negligible, the formation of ethane would have to be virtually completely suppressed.

If the earlier data, obtained by the pyrolysis of di-tertiary-butyl peroxide in the presence of isobutane (1), can be represented by a mechanism composed of reactions [*l*] and [*m*],

$$\text{DTBP} \rightarrow 2(CH_3)_3CO \quad [l]$$

$$(CH_3)_3CO \rightarrow CH_3 + CH_3COCH_3, \quad [m]$$

along with [*a*], [*c*], [*d*], [*e*], [*f*], and [g], an expression may be obtained which is identical in form with m_{IV}. However, in this application $\lambda_E = 2R_{C_2H_6}/R_{CH_3COCH_3}$. Acetone was not determined in those experiments but its rate of formation may be calculated with sufficient accuracy from the Arrhenius equation for the decomposition of the peroxide. Figure 1 also shows these data, in the original units, and indicates that application of the extended mechanism, with $\beta = 0.446$, gives essentially constant values of $k_c/k_a^{1/2}$. This is in contrast to the curvature observed in $R_{CH_4}/R_{C_2D_6}^{1/2}[i\text{-}C_4H_{10}]$ also shown in Fig. 1.

It is useful to note how the systematic error which arises from the neglect of reactions

of the type [*d*] varies, through the value of β, with various hydrocarbons. Values of m_{IV} were calculated for $\beta = 0.057$ ($k_d/k_e = 0.06$ as found (7) for $CH_3 + C_2H_6$) and $\beta = 0.18$ ($k_d/k_e = 0.22$ as found (4) for $CH_3 + sec\text{-}C_3H_7$), and are shown in Fig. 2 as curves B and C respectively. The very much lower values of β encountered in these systems allow the taking of data without significant error at considerably larger values of λ_E.

The question also arises as to what effect neglect of the disproportionation reaction [*d*] has on the activation energy of the hydrogen-transfer reaction [*c*] when $m < 1.0$. It becomes immediately obvious when equation [2] is rewritten as follows that, provided m is kept independent of the temperature, the apparent and true activation energies will be identical.

$$\rho = m^{-1}(R_{CD_3H})_c/R^{1/2}_{C_2D_6}[i\text{-}C_4H_{10}] = (k_c/k_a^{1/2})_{app} \qquad [10]$$

If, owing to a decrease in λ_E, m increases with increasing temperature, the apparent value will be less than the true value, but the difference will be small in most instances. For example, it will be approximately 0.5 kcal mole^{-1} if m increases by 0.20 between 100° C and 250° C; this is comparable to the uncertainty often quoted in determinations of activation energy.

In conclusion, the variation with isobutane pressure of ρ (or $R_{CH_4}/R^{1/2}_{C_2D_6}[i\text{-}C_4H_{10}]$ in the DTBP system) can be accounted for by assuming the disproportionation reaction [*d*] to be a significant source of methane in these systems under some conditions. It is possible to relate the contribution of [*d*] to the known or measurable quantities β, λ_H, λ_D, and λ_E and thus to correct the data for that contribution. Some assumptions were necessary in the development of the relevant expressions but it is doubtful that the quantitative conclusions are seriously in error.

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APPENDIX I

The referee of the present paper has asked us to comment on our use of [3] rather than [3*a*], the analogue of which occurs frequently in the literature.

$$k_e/k_a^{1/2}k_g^{1/2} = 2 \qquad [3a]$$

Kerr and Trotman-Dickenson (4, 9) and Trotman-Dickenson (10) have cited several systems in which [3*a*] appears to be verified experimentally.

Perhaps the simplest discussion can be given if the more general case is considered.

$$R_1 + R_1 \rightarrow \text{alkane}_1 + \text{alkene}_1 \quad [A]$$

$$\rightarrow R_1R_1 \quad [B]$$

$$R_1 + R_2 \rightarrow \text{alkane}_1 + \text{alkene}_2 \quad [C]$$

$$\rightarrow \text{alkene}_1 + \text{alkane}_2 \quad [D]$$

$$\rightarrow R_1R_2 \quad [E]$$

$$R_2 + R_2 \rightarrow \text{alkane}_2 + \text{alkene}_2 \quad [F]$$

$$\rightarrow R_2R_2 \quad [G]$$

The formulations corresponding to [3] and [3*a*] are, respectively,

$$(k_C+k_D+k_E)/(k_A+k_B)^{1/2}(k_F+k_G)^{1/2} = 2, \quad [i]$$

and

$$k_E/k_B^{1/2}k_G^{1/2} = 2. \quad [ii]$$

[A] and [D] are not observed when R_1 is a methyl radical.

It should be emphasized that [i], and thus [3], is the correct statistical formulation provided that each collision results in either disproportionation or combination of the colliding pair of radicals. The latter assumption, of course, underlies all the development in the body of this paper. If the steric factor is much less than unity for the sum of mutual disproportionation plus combination for some radical species, no theoretical relationship exists when a methyl radical is taken with that radical in a cross combination; this follows from the fact that the steric factor for the combination of methyl radicals is very close to unity (11). In that case, then, there is no a priori reason to expect either [i] or [ii] to hold.

If [i] is a correct formulation and the experimental data lead to [ii], it follows that the right side of [iii] must very nearly be unity.

$$(1/2)k_E/k_B^{1/2}k_G^{1/2} = [1+(k_B/k_A)]^{1/2}[1+(k_F/k_G)]^{1/2}/[1+(k_C/k_E)+(k_D/k_E)] \quad [iii]$$

Data are available to check [iii] for the systems tabulated.

R_1	R_2	k_A/k_B	k_F/k_G	k_C/k_E	k_D/k_E	[iii]
CH_3	C_2H_5	0	0.12*	0	0.06†	1.0
CH_3	n-C_3H_7	0	0.16‡	0	0.14§	0.94
CH_3	n-C_4H_9	0	0.94‖	0	0.15‖	1.21
C_2H_5	i-C_3H_7	0.12*	0.60¶	0.43§	0.06**	0.90

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**Calculated from $k_D/k_C = 0.15$ and $k_C/k_E = 0.43$ given in footnote §.

Thus, within some 20%, the cancellation to unity is observed. This uncertainty is not much greater than that of the experimental values reported for $R_1R_2/(R_1R_1)^{1/2}(R_2R_2)^{1/2}$ used to support the use of [ii] and [3*a*].

It should be noted that the values of k_C/k_E reported in reference 4 for the systems CH_3, i-C_3H_7 and CH_3, t-C_4H_9 cannot be used to check [iii], since those values were obtained indirectly in that the equivalent of [ii] was used to calculate unmeasured products. The

ratios of rate constants reported by Boddy and Robb (12) were not considered here; reasons for questioning the reliability of those data have been advanced elsewhere (4, 10).

APPENDIX II

A brief description is given of the derivation of the expressions for m which appear in the text as equations [4], [5], [6], and [7]. To simplify the nomenclature, the following abbreviations are used: $[CD_3COCD_3] = a$, $[i\text{-}C_4H_{10}] = b$, $[CD_3] = v$, $[t\text{-}C_4H_9] = u$, $[CD_3COCD_2] = w$, and $\sigma = v+u+w$.

The balances for v, w, and u respectively are:

$$2R_{CO} = 2k_a v^2 + k_b av + k_c bv + (k_d+k_e)uv + k_h wv \qquad [i]$$

$$R_{CD_4} = k_b av = k_h vw + k_i uw + 2k_j w^2 \qquad [ii]$$

$$mR_{CD_3H} = k_c bv = (k_d+k_e)uv + 2(k_f+k_g)u^2 + k_i uw. \qquad [iii]$$

If it is assumed that

$$2k_a = 2(k_j+k_g) = 2k_j = (k_d+k_e) = k_h = k_i = k, \qquad [3]$$

the addition of [i], [ii], and [iii] and substitution of [3] yields [iv].

$$\sigma = (2R_{CO}/k)^{1/2} \qquad [iv]$$

Combination of equations [ii] and [iv] gives

$$w = \sigma R_{CD_4}/2R_{CO} = \sigma\lambda_D. \qquad [v]$$

Similarly, from equations [iii] and [iv],

$$u = m\sigma R_{CD_3H}/2R_{CO} = m\sigma\lambda_H. \qquad [vi]$$

Because $(2R_{C_2D_6})^{1/2} = k^{1/2}v$, [iv] may be used to show that

$$v = \sigma(R_{C_2D_6}/R_{CO})^{1/2} = \sigma\lambda_E^{1/2}. \qquad [vii]$$

Rearrangement of the relation

$$R_{CD_3H} = k_c bv + k_d uv = mR_{CD_3H} + k_d uv$$

and application of equation [iv] yields the following expression for m:

$$m = 1-(\beta uv/\sigma^2\lambda_H), \qquad [viii]$$

where

$$\beta = k_d/(k_d+k_e). \qquad [ix]$$

However,

$$u = \sigma-v-w = \sigma(1-\lambda_D-\lambda_E^{1/2}), \qquad [x]$$

and the expression for m_I is obtained by substitution of [x] into [viii]. Similarly,

$$v = \sigma(1-\lambda_D-m\lambda_H), \qquad [xi]$$

and, substituting in [viii], the expression for m_{II} is obtained. Substitution of uv in [viii] by

$$uv = m\sigma^2\lambda_H\lambda_E^{1/2} \qquad [xii]$$

gives the relation for m_{IV}. In addition, [iii] can be rewritten using [x] as

$$mR_{CD_3H} = k\sigma u = k\sigma^2(1-\lambda_D-\lambda_E^{1/2}).$$

The expression for m_{III} follows after substitution of [iv].

If any pair of the relations for m are equated, the same expression for β is obtained. The manipulation is easy except when m_{II} is one of the expressions in the pair taken. Furthermore, substitution of this expression for β into the relations for m_I, m_{II}, or m_{IV} yields that for m_{III}. This provides good evidence for the existence of the algebraic identity $m_I = m_{II} = m_{III} = m_{IV}$. Consequently, when calculating values of m the relation which gives the most precise result can be used.

Finally, because λ_E tends to unity as light intensity is increased and isobutane pressure is decreased, it can be shown that m will tend to $1/(1+\beta)$ at high light intensity and low isobutane pressure and that m will tend to unity under the opposite conditions, i.e. $\lambda_E \rightarrow 0$ (equation [7]).

DESIGN AND CALIBRATION OF AN INEXPENSIVE FREE-FLOW ELECTROVISCOMETER[1]

P. A. D. DE MAINE AND E. R. RUSSELL

ABSTRACT

Here are reported the design and calibration of a new inexpensive free-flow electroviscometer for rapid and accurate measurement of the viscosity coefficients for conducting and non-conducting solutions. Flow times of 30 seconds are easily reproduced to within 0.02 second.

INTRODUCTION

In the past, several comprehensive review articles about viscometers have been published (1–5). An automatic viscometer designed to measure flow times accurately without constant attention by the operator has been reported by Goldfinger and Greatbatch (6). This viscometer used a photoelectrically triggered timing device to measure flow times to within 1 part in 10,000. Stock (7) has designed an electric resistance stream gauge type viscometer.

Measurements with free-flow type viscometers are limited in accuracy by the response time of the observer and the flow time of the viscometer. Rotary cone and the Goldfinger–Greatbatch types are relatively expensive and create problems where temperature control is desired.

Here is described a new modification of the Ostwald–Fenske viscometer which can be immersed in the usual thermostated bath. Even the low flow times, less than 30 seconds, are measured electronically to better than 1 part in 1000.

DESCRIPTION OF APPARATUS

Electrodes of No. 24 gauge platinum were sealed into an Ostwald–Fenske viscometer at the locations shown in Fig. 1. Platinum-to-glass or Hysol cement seals are satisfactory. Special care should be taken to locate the lowest electrode (No. 3) away from the capillary entrance. The top two electrodes (Nos. 1 and 2) are located centrally to minimize holdback of the solution. Lead wires are soldered to the three electrodes and then are coated with Dupont Duco cement for electrical insulation. Cover assemblies were cut from solid Plexiglass and were cemented directly onto the viscometer with Dupont Duco cement. These cover assemblies fit into openings in the lid of the Precision Temp-trol kinematic viscosity bath (Precision Scientific Company, Chicago, Illinois), and thus correct positioning of the viscometer is assured at all times.

Two Model 30 Fisher transistor relays (Fisher Scientific Company, New York, N.Y.) were connected to the platinum electrodes on the viscometer (Fig. 2) through variable resistances (0 to 0.50 meg) and to a Precision Time-it timer (Precision Scientific Company, Chicago, Illinois) graduated in 1/10 seconds. Viscosity measurements at constant, desired temperatures were achieved by placing the viscometers in the Temp-trol kinematic viscosity bath or in a similar laboratory-constructed unit.

OPERATION AND CALIBRATION

Methanol – carbon tetrachloride solutions of $SnCl_4.5H_2O$ and methanol solutions of iron group metal chlorides were used in the calibration of the modified Ostwald–Fenske

[1]*Manuscript received April 10, 1961.*
Contribution from the Department of Chemistry, University of Mississippi, University, Miss., U.S.A.

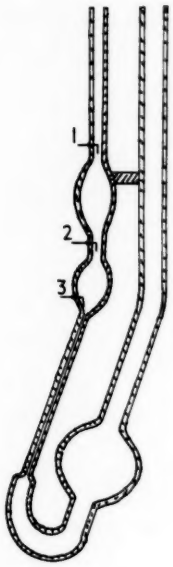

FIG. 1. Diagram of the modified Ostwald–Fenske viscometer showing location of the No. 24 gauge platinum electrodes.

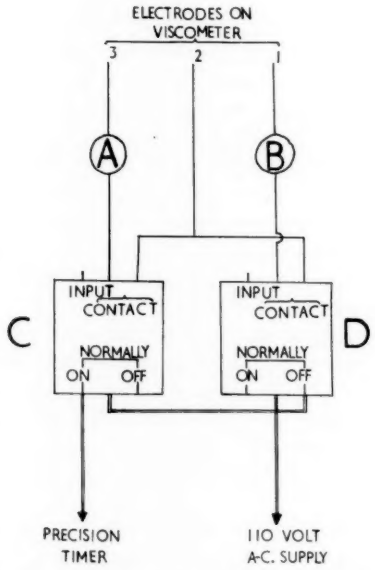

FIG. 2. Electrical circuit diagram for the electroviscometer. A and B: variable resistances (0 to 0.5 meg), graduated in 10,000-ohm units; C and D: Fisher transistor relays with switches in "delay off" and "NC" positions.

viscometers. $CaCl_2$, $CrCl_3.6H_2O$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$, $CoCl_2.2H_2O$, $NiCl_2.6H_2O$, $NiCl_2.2H_2O$, $CuCl_2.2H_2O$, and $ZnCl_2$ were used. Accurate viscosity data for these solutions as measured with normal Ostwald–Fenske viscometers at 20° C or 25° C and 45° C have been reported (8, 9). It has also been shown that molar conductance values for these solutions are in the range 0.80 to near 160 (10, 11, 12). Densities accurate to four decimal places have also been reported (8, 12).

Ten milliliters of solution was pipetted into the electroviscometer. The resistance (R) between electrodes 1 and 2 or between 2 and 3 (Fig. 1) with the viscometer immersed in the thermostated bath, and the immediate change in resistance (ΔR) as the solutions flowed past the two top electrodes, were measured directly with a Simpson Avometer. The maximum resistance ($\bar{R}$) required to operate the contact circuits for each of the two relays, C and D (Fig. 2), was determined by varying resistances A and B respectively. For the four relays used in this work, $\bar{R}$ lies between 200,000 and 310,000 ohms. The variable resistances A and B were set at a value $\mathring{R}$ so that $\mathring{R}+\Delta R = \bar{R}+10{,}000$. Experiments have shown that for all the conducting solutions studied, $\mathring{R}$ can be set at any value between 50,000 and ($\bar{R}-20{,}000$) ohms. $\bar{R}$ usually lies between 200,000 and 310,000 ohms. Values for $\mathring{R}$ between these limits do not give any change in flow-time values.

The solution was drawn up in the viscometer so as to cover electrodes 1, 2, and 3. As the falling solution passed the top electrode the timer was switched on automatically and then the timer was switched off as the liquid passed the second electrode. Typical data collected for solutions of $SnCl_4.5H_2O$ are given in Table I. The factor limiting the accuracy appears to be the 0.1-second units (readable to 0.05 second) on the timer.

TABLE I

Flow rates for solutions of composition indicated, at 25° C, as measured with the electroviscometer

$SnCl_4.5H_2O$ (concn., M)	CCl_4 (concn., M)	Time (sec.)	No of trials	SRMS
0.100	0.000	40.17	7	0.02
0.200	0.000	43.37	7	0.02
0.300	0.000	46.81	7	0.03
0.400	0.000	50.55	10	0.03
0.500	0.000	54.88	7	0.03
0.600	0.000	59.07	8	0.04
0.700	0.000	63.63	9	0.03
0.800	0.000	68.71	7	0.02
0.900	0.000	74.11	7	0.02
1.000	0.000	79.97	6	0.03
0.100	1.029	39.72	7	0.02
0.100	2.057	39.23	6	0.03
0.100	3.086	39.01	8	0.03
0.100	4.114	38.84	7	0.02
0.100	5.143	38.49	8	0.02
0.100	6.171	38.27	9	0.02
0.100	7.200	38.57	7	0.03
0.100 / 0.100	Two-layer formation*			

NOTE: Average times listed are for the solution to flow from the first electrode to the second electrode. Square root mean square (SRMS) deviations and the number of independent measurements made are also given. For each solution the maximum deviation did not exceed 0.05 second.

*P. A. D. de Maine and M. M. de Maine. J. Inorg. & Nuclear Chem. **14**, 142 (1960).

In Figs. 3 and 4 are plotted viscosity coefficients, determined with the usual Ostwald–Fenske viscometer (flow time 400 to 1100 seconds), versus the product of density (8, 12)

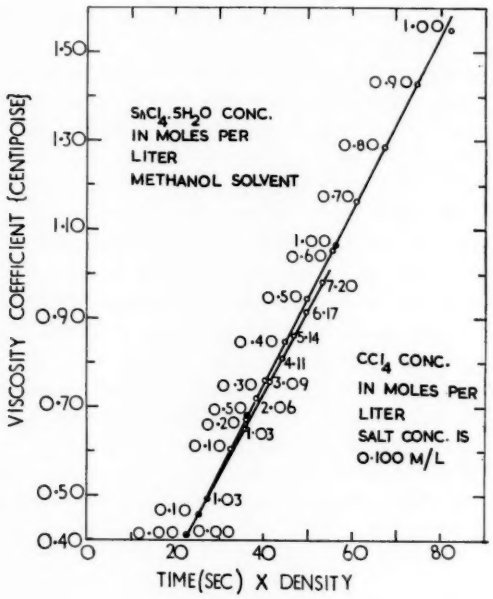

FIG. 3. Plots of viscosity coefficient, determined with normal Ostwald–Fenske viscometers, versus the product of density × time, measured with the electroviscometer, for $SnCl_4.5H_2O$ dissolved in pure methanol or in methanol – carbon tetrachloride: ○ 25° C; ● 45° C. (Except for the 1.000 *M* $SnCl_4.5H_2O$ solution, the maximum deviation from each straight line is near 1 part in 1000.)

and flow time (in seconds) measured with the new electroviscometer. The linear relations (maximum deviation 0.10%) found for the methanol solutions (Figs. 3 and 4) indicate that the flow characteristics of the modified Ostwald–Fenske viscometer do not change significantly with temperature or with the viscosity of the solution. For $SnCl_4.5H_2O$ solutions, similar linear relations exist for each solvent composition studied. The deviation for the plot for systems of high carbon tetrachloride concentration is just 1% from the plot for solutions with pure methanol as solvent (Fig. 3). This deviation probably arises from the gross changes in the surface tension and density as one passes from pure methanol to methanol – carbon tetrachloride, with the latter of high concentration.

Thus with calibration plots for each solvent and a modified Ostwald–Fenske viscometer, the viscosity coefficient of conducting solutions can be rapidly and accurately determined at any temperature. In our laboratory 200 measurements for 20 different solutions at two temperatures were made in less than 6 hours. Moreover, this inexpensive electroviscometer has been adapted to measure flow times for non-conducting liquids by addition of small amounts of a conducting compound, by use of higher resistances in parallel, or by different spacing of the electrodes.

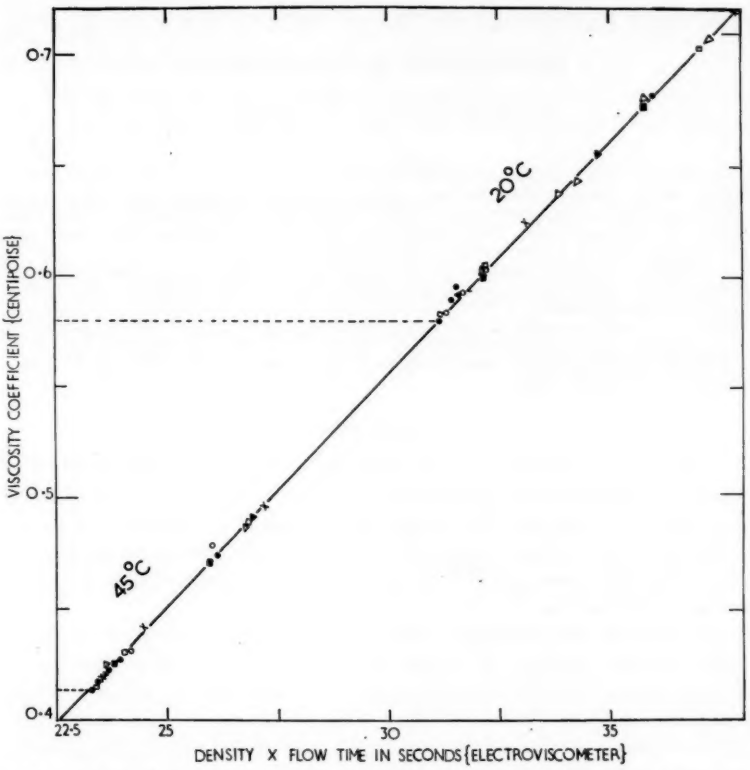

FIG. 4. Plots of viscosity coefficient, determined with normal Ostwald–Fenske viscometers, versus the product of density×time, measured with the electroviscometer, for salts dissolved in pure methanol: ○ $CaCl_2$; □ $CuCl_2.2H_2O$; × $NiCl_2.6H_2O$; ● $NiCl_2.2H_2O$; ■ $CoCl_2.6H_2O$; △ $CoCl_3.2H_2O$; ▲ $CrCl_3.6H_2O$; – – – viscosity coefficient for pure methanol. (Only 36 of the 66 points falling on the straight line are shown.)

ACKNOWLEDGMENT

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HYDROGEN EXCHANGE BETWEEN CELLULOSE AND WATER

I. MEASUREMENT OF ACCESSIBILITY[1]

O. SEPALL[2] AND S. G. MASON

ABSTRACT

Estimates of the extent of the hydrogen exchange reaction between cellulose and water vapor determined by an improved method using tritiated water and by the use of heavy water were usually in good agreement. With certain samples, surface roughness had a small effect upon the measurement of tritium radioactivity in cellulose.

The accessibility or limiting extent of the exchange increased slightly with relative humidity in the range 20 to 100% and was attributed to the reduction of crystalline order reported recently in similar experiments.

In contrast to cellulose whose accessibility corresponds approximately to the amorphous fraction, a partially crystalline amylose was 100% accessible while an amorphous xylan was 52% accessible, indicating that in these substances the accessibility and the fraction of amorphous material are not identical.

INTRODUCTION

Although valuable contributions to the knowledge of the fine structure of cellulose have been made by studies of the hydrogen exchange reaction with water (1, 2, 3), work based on this technique has not been extensive because the experimental methods are time consuming and of limited applicability. This paper describes measurements of exchange using tritiated water (HTO) by an improved method which permits greater scope in studies of the reaction.

In most investigations, the exchange of deuterium with D_2O has been measured either by determining isotopic dilution (3) or the infrared absorption of deuterated and unreacted hydroxyl groups (1). The present results were obtained by the tritium exchange method devised by Lang and Mason (2) but using an improved apparatus (4) which is simpler and gives a more precise measurement. To calibrate the method and corroborate the results, the exchange of deuterium with pure D_2O was also determined by infrared (1) and gravimetric (5) techniques.

The accessibility, A^0, defined as the limiting extent of the exchange, was determined for various cellulose samples, starch, and xylan. The influence upon A^0 of the crystallinity of the sample and relative humidity was also investigated in a preliminary study of the factors governing hydrogen exchange in cellulose and similar polymers.

EXPERIMENTAL PART

Materials

The samples listed below were used.

1. Amylopectin: commercial grade, manufactured from potato starch from Stein Hall and Co.

2. Amylose: as for sample 1.

3. Cellophane: air-dried viscose film without additives, supplied by Dupont of Canada Ltd.

4. Wood cellulose: softwood, acetate grade wood pulp; 96% alpha cellulose (TAPPI method).

[1]*Manuscript received June 14, 1961.*
Contribution from the Physical Chemistry Division, Pulp and Paper Research Institute of Canada, and the Department of Chemistry, McGill University, Montreal, Que.
[2]*Holder of a Canadian Industries Limited Fellowship, 1960–61.*

5. Bacterial cellulose: kindly supplied by Dr. J. R. Colvin of the National Research Council, Ottawa.

6. Cotton linters: dewaxed by alcohol–benzene extraction.

7. Commercial paper grade woodpulps by Anglo Paper Products Ltd., Quebec, Que.

8. Xylan: birch (*Betula papyrifera*) xylan prepared by the method of Glaudemans and Timell (6).

9. Milled wood cellulose: sample 4 milled in an Intermediate Model Wiley Cutting Mill using three passes with a 150-mesh screen.

Methods

1. Deuterium Exchange: Gravimetric (A_1)

The method is based on that of Morrison (5) and involves determining the increase in mass of the sample following exchange with pure D_2O.

The apparatus used (Fig. 1) consisted of a 100-ml Pyrex flask (G) with two outlets

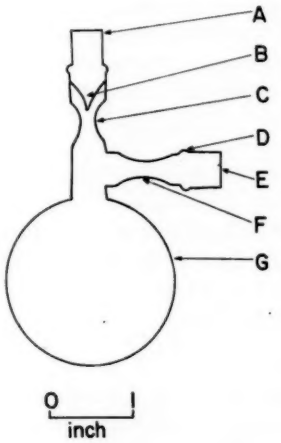

FIG. 1. Cell for gravimetric measurement of deuterium exchange. A, outlet to fit Veeco Quick Vacuum Coupling (No. C-50); B, break-seal; C and F, constrictions for sealing; D, expanded section for vacuum seal with O-ring; G, 100-ml flask.

(A and E) of suitable diameter to fit a Veeco Quick Vacuum Coupling on a vacuum system. The expanded section in the outlet as at D helped to form the vacuum seal created by an O-ring. The vessel could be sealed by fusing at the constrictions C and F. The break-seal (B) eliminated the need for a stopcock.

About 6 g of sample was placed in the flask of tared weight W_1 and dried by evacuating through outlet E for 48 hours at 75° C and at 1 μ Hg vacuum. The flask was sealed and weighed (W_2). The break-seal was opened by inserting a steel rod in A, and the apparatus shown in Fig. 2 was used to react the sample with pure D_2O. Dry air, obtained from the evaporation of liquid air in a Dewar flask shown at H in Fig. 2, was bubbled through liquid D_2O in J at constant temperature and then conducted into the sample in flask L (immersed in a thermostat) by a stainless steel tube (K). After the desired reaction time, the sample was dried by evacuating the flask for 48 hours at 75° C and at 1 μ Hg vacuum.

The flask was weighed (W_3) and the difference in weight ($W_3 - W_2$) was corrected for

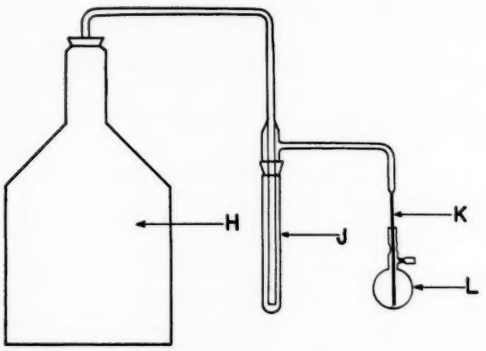

FIG. 2. Apparatus for conducting exchange with D_2O. H, Dewar flask with liquid air; J, vessel containing D_2O liquid; K, stainless steel tube; L, vessel shown in Fig. 1.

changes in air buoyancy to give the increase of mass ΔW corresponding to exchange of hydrogen by deuterium. The mass S of the sample was determined similarly from $(W_2 - W_1)$. The percentage of accessibility A_1 was calculated from

$$[1] \qquad A_1 = 100(\Delta W)/F \times S.$$

The factor F is the fractional increase in mass of the same substance after the hydroxyl hydrogens have been completely exchanged by deuterium. For cellulose and starch, $F = 0.0185$; for birch xylan, F was calculated to be 0.0140, assuming a linear chain of β-D-xylopyranose residues, linked by 1,4-glycosidic bonds and having a single terminal side chain of the potassium salt of 4-O-methyl-α-D-glucuronic acid attached glycosidically to C_2 of every 11th xylose residue (7).

No correction for the isotopic purity of the D_2O (99.7%) was necessary.

In contrast to Morrison's experiments (5), the exchange was conducted at constant relative humidity. This was done to avoid the partial reaction with inaccessible hydroxyl groups, shown to occur if the sample moisture was varied during the exchange (8).

The results reported are for single measurements only and were reproducible to 1%.

2. Tritium Exchange: Radioactivity (A_2)

The method used for measuring tritium exchange between cellulose and tritiated water (HTO) vapor has already been described (4), and consisted of (*a*) exposing the sample to tritiated water vapor, (*b*) drying, and (*c*) determining tritium radioactivity in the sample using a methane-filled, windowless proportional counter of special design in which all three operations were carried out.

During the treatment with tritiated water vapor, the relative humidity (RH) was kept constant to ±3% by suitably controlling the temperature of the liquid-HTO reservoir, and the sample temperature, to ±0.5° C. The samples were dried at 75° C in a vacuum of 1 μ Hg for 15 hours to remove sorbed water (2).

The samples were used in sheet form of superficial density of about 3 mg/cm².

Amylose was dissolved in water at 160° C and a film was deposited from the solution on a polished, chromium-plated surface by drying at room temperature. An aqueous amylopectin solution was prepared at room temperature, and, since the unsupported film tended to crack during drying, it was deposited onto a cellophane supporting film. Handsheets were prepared from fibrous materials such as wood cellulose by the TAPPI

procedure which consisted of (*a*) depositing the wet sheet of fibers from a water suspension on a fine screen, (*b*) pressing to remove moisture, and (*c*) drying the sheet at room temperature in contact with a polished, chromium-plated surface. The cotton linters required a preliminary beating before a satisfactory handsheet could be formed; this was done in a PFI Laboratory mill. Cellophane film was used as received.

The accessibility A_2 was determined from the relation

$$[2] \qquad A_2 = \frac{Rf}{c}\left(\frac{c_0}{R_0 f_0}\right) A_{1,0},$$

where R is the sample count rate; c, the activity of the tritiated water (about 1.5 μc/mg in these experiments); and f, the vapor/liquid partition ratio of tritium at the temperature of the liquid-HTO reservoir. The factor f is a known correction factor for the principal isotope effect (9). Quantities with the subscripts "0" are those of a standard sample whose accessibility $A_{1,0}$ was determined by the gravimetric D_2O exchange method under the identical conditions of temperature and relative humidity used in measuring R_0. Cellophane at 75% relative humidity and at 25° C was used as the standard sample.

The results are averages of four determinations on cellophane, and of two on wood cellulose and other materials; they were reproducible to 1%.

The principal difference between A_2 and the quantity A_s measured by Lang and Mason (2) was that the samples were dried rigorously after the exchange reaction by thoroughly evacuating at 75° C instead of room temperature. In addition, the precision of R was greater.

3. Deuterium Exchange: Infrared (A_3)

The accessibility of amylose and amylopectin was estimated by the infrared absorption method used by Marrinan and Mann for deuterated cellulose (1). An infrared cell (Fig. 3) suitable for investigating evacuated samples was constructed for use with the

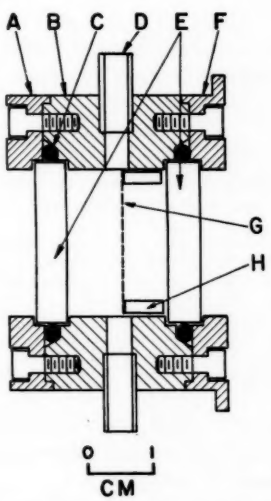

FIG. 3. Vacuum infrared absorption cell. A, front cover (stainless steel); B, cell body (stainless steel); C, O-ring; D, D_2O vapor circulation line; E, calcium fluoride windows; F, back cover to fit mounting on Perkin–Elmer model 21; G, sample film; H, annular sample holder.

Perkin–Elmer model 21 spectrometer. Samples were inserted by removing cover plate A, which was sealed by O-rings to the standard calcium fluoride windows and to the body (B) of the cell. D_2O vapor was circulated through tube D, which was sealed by vacuum valves (not shown).

Sample films having a superficial density of 0.8 mg/cm² were prepared by evaporating aqueous solutions of amylose and amylopectin on a mercury surface. The film (G in Fig. 3) was attached to the annular sample holder (H) by a small amount of vacuum grease.

The sample film was reacted with D_2O vapor while in the absorption cell. A stream of air containing the vapor was produced with the arrangement shown in Fig. 2 and was circulated through the cell for 8 hours. The cell was evacuated for an hour to remove most of the sorbed water and sealed.

The absorbance was measured at $\nu_1 = 2530$ cm^{-1}, corresponding to an absorption peak for the OD group, and at $\nu_2 = 3360$ cm^{-1}, for OH (1). Assuming Beer's law to apply

$$[3] \qquad \frac{\log (I_0/I)\nu_1}{\log (I_0/I)\nu_2} = \frac{k_{OD}\, c_{OD}}{k_{OH}\, c_{OH}} = 1.11\, \frac{c_{OD}}{(1-c_{OD})}$$

$$[4] \qquad = 1.11\, \frac{A_3}{1-A_3}.$$

I_0 and I are the intensities of the incident and transmitted radiation; c is the mole fraction of absorbing groups and k the extinction coefficient; A_3 ($= c_{OD}$) is the corresponding accessibility. It was assumed that in starch $k_{OD}/k_{OH} = 1.11$, the same value used for cellulose (1), so that A_3 could be evaluated by equation [4].

As in the gravimetric measurement, the correction for isotopic purity of the heavy water was neglected.

RESULTS

Reaction Time

The time required to achieve limiting values of A_1 and A_2 was investigated at 75% RH and 25° C for cellophane and wood cellulose. The results given in Table I show that A_1

TABLE I

Effect of reaction time upon A_1 and A_2 (75% RH, 25° C)

Sample	Reaction time (days)	A_1 (%)	Sample	Reaction time (hours)	A_2 (%)
Cellophane	2	60.5	Wood cellulose	1	50.8
,,	4	78.5	,,	2	54.6
,,	4	79.0	,,	4	57.0
,,	6	78.0	,,	8	58.0
			,,	32	58.3

was approximately constant after 4 days and A_2, after about 8 hours. The long time required for deuterium exchange was undoubtedly due to the low rate of transfer of D_2O to the sample in the presence of air.

The influence of relative humidity in the range 20 to 75% RH upon the time required for A_2 to approach a limit A_2^0 was investigated for cellophane. Values of A_2 for different reaction times are plotted in Fig. 4 and show that after the initial rapid reaction reported

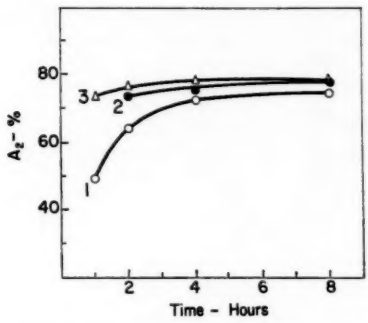

FIG. 4. Tritium exchange versus time for cellophane in HTO vapor. Curves 1, 2, and 3 correspond to relative humidities of 20, 50, and 75% respectively at 25° C.

earlier (1), the extent of the exchange tended to an asymptotic limit after about 8 hours. The reaction was slower at lower relative humidity and A_2 approached a smaller value. The decreased rate of reaction was probably due to slower diffusion of water vapor in cellulose at the lower moisture content (10). The lower limiting exchange, indicating that fewer reactive hydroxyls were available, is discussed later.

For amylopectin, a limiting value of $A_2^0 = 98\%$ at 75% RH and 25° C was also found after 8 hours. It was assumed that the rate of exchange with the other cellulose samples was similar to that with cellophane and wood cellulose.

For measurements of the limiting extent of the exchange denoted by the superscript "0", at least 4 days' exposure to D_2O was allowed in measuring A_1^0 and at least 8 hours' exposure, in measuring A_2^0. For A_3^0, an exposure of 8 hours was used, although it has been shown that little further reaction is detected in cellulose film after 1 hour in D_2O vapor (1).

Comparison of A_1^0, A_2^0, and A_3^0 for Different Samples

Values of A^0 were measured by the different methods for a number of samples at 75% RH and 25° C (Table II). The differences between A_2^0 and measurements of A_3 on

TABLE II

Comparison of methods of measuring A (75% RH, 25° C)

Sample	A_1^0 (%)	A_2^0 (%)	A_3^0 (%)
Cellophane	79	(79)*	
Amylopectin	96	98	97
Amylose	99	95–98	98
Wood cellulose	58	58	
Bacterial cellulose	42	39	
Cotton linters	40	44	
Birch xylan	52		
Milled wood cellulose	76		

*Assumed for purposes of calibration.

similar samples of Lang and Mason (2) ranged from 2 to 8% and were probably due to the slightly different experimental conditions employed.

In most cases, A_1^0 and A_2^0 were in good agreement (Fig. 5), except for +4% difference

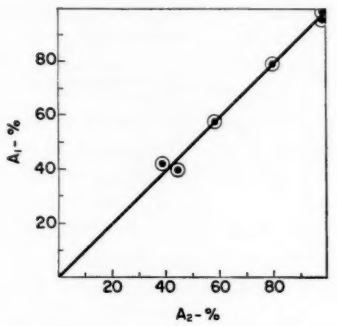

FIG. 5. Comparison of deuterium exchange (gravimetric) and tritium exchange methods of measuring A^0 (75% RH, 25° C).

for cotton linters and -3% for bacterial cellulose. It is believed that these discrepancies arose from surface roughness of the samples. The influence of surface roughness upon the measurement of tritium in tritiated cellulose, considered by calculating counting rates for simple models for the samples listed in Table II, is discussed elsewhere (11). It was concluded that the shape of surface irregularities influenced the measurement while the bulk density had negligible effect; the analysis made it possible to account for the differences observed here. The error can be avoided by using smooth samples or by independently calibrating the tritiation method for each material.

The high accessibility of amylose and amlyopectin was confirmed by the three methods. It is possible that here $A^0 = 100\%$, but that the measured values were influenced by factors such as (*a*) impurities in the samples, (*b*) deviations from Beer's law, and (*c*) contamination of D_2O by atmospheric moisture during the exchange reaction. Limited exchange in fully deuterated amylose and amylopectin from potato starch has recently been reported from infrared absorption measurements (12). The extent of the reaction was measured by the ratio (area of OD absorption band)/(area of OH absorption band), which was about 2.6 for both deuterated samples (12), while in the present measurements, the ratio was 55 for deuterated amylose. The reason for the difference is not clear; possibly the method of isolation can affect the properties.

Only A_1^0 was measured for xylan and milled wood cellulose because contact with water, as during preparation of sheet samples, has been found to produce recrystallization in similar materials (13). Milling increased A_1^0 as expected since a similar treatment has been shown to increase the amorphous fraction in cellulose determined by X-ray diffraction (13). The crystallinity parameter K_{002} (13) was found to be 1.25 for the original material and 1.13 for the milled samples, indicating some destruction of crystalline regions. The radial photometer traces for the samples showed that the crystallinity was not entirely destroyed by milling; this is presumably the reason for A_1^0 being less than 100%.

X-ray diagrams of amylose exhibited distinct diffraction rings while those of birch gave a diffuse halo, indicating that the former was partially crystalline and the latter wholly amorphous. The results were unexpected because complete accessibility of amylose, by analogy with cellulose, implied the absence of crystallinity. Evidently the analogy is not necessarily valid and other factors which are presently unknown determine the accessibility of amylose and xylan.

Relative Humidity

Values of A_2^0 were measured for wood cellulose and cellophane from 0.6 to 100% RH and at 25, 50, and 100° C. The results, summarized in Table III, indicate that A_2^0, at

TABLE III

Effect of relative humidity upon the accessibility of cellulose

Sample	Temp. (°C)	RH (%)	A_1^0 (%)	A_2^0 (%)
Cellophane	25	20		76
,,		50		79
,,		75	79	(79)*
,,		95		80
,,		100		80
Wood cellulose	25	20	56	55
,,		50		57
,,		75	58	58
,,		85		60
,,		95		61
,,		100		62
,,	50	25		58
,,		75		57
,,	100	0.6		67
,,		3		66
,,		10		68
,,		25		64

*Assumed value for purposes of calibration (eq. [2]).

25° C, of wood cellulose, and to a lesser extent of cellophane, increased slightly with increasing relative humidity. At 50 and 100° C the results appeared to be unaffected by relative humidity. It is not known whether the higher values at 100° C indicate a higher accessibility or a temperature-dependent isotope effect in the partition of tritium between HTO vapor and the hydroxyl groups in cellulose. Attempts to measure A_1^0 at 100° C were unsuccessful mainly because no satisfactory correction could be made for the loss in mass of cellulose occurring during contact with air under these conditions. The decrease in mass may have resulted from chemical degradation which has been observed in cotton linters as a reduction of degree of polymerization and of carboxyl groups after only 20 minutes in air at 100° C (14).

There is a possible error in the measurements of tritium exchange at different relative humidities because the tritium is concentrated in the residue during evaporation as in drying the sample. However, the effect was probably small because drying was rapid and the equilibrium separation would not have occurred. The higher values of A_2^0 with increasing relative humidity are therefore believed to indicate a greater number of reactive hydroxyls. The significance of the result is discussed below.

Comparison of Commercial Woodpulps

Values of A_2^0 were measured for commercial woodpulps prepared by alkaline (kraft and soda) and sulphite processes from Canadian softwoods and hardwoods. Since the samples also contained lignin and hemicelluloses in addition to cellulose, the values of A_2^0 are not necessarily the percentage of hydroxyl groups reacted. The results indicate only the relative abundance of reactive hydroxyls in the different samples compared with cellulose.

The results (Table IV) show only small differences between the samples. Wood fibers

TABLE IV

Comparison of commercial woodpulps

Sample	A_2^0 (%)
Bleached softwood sulphite	59.0
Bleached softwood kraft	60.0
Bleached hardwood kraft	57.5
Bleached hardwood soda	57.5
Unbleached softwood sulphite (Permanganate No. 10)*	58.0
Unbleached softwood sulphite (Permanganate No. 20)	57.5
Unbleached softwood sulphite (Permanganate No. 30)	55.5

*TAPPI method. (The permanganate number is a measure of lignin content and is approximately zero in bleached pulps.)

with higher lignin content appeared to have fewer accessible hydroxyls, as would be expected, since the hydroxyl content of lignin is lower than that of cellulose. The slightly lower values for hardwood fibers may have similarly indicated a higher content of xylan.

DISCUSSION

The results demonstrate the validity, versatility, and convenience of the tritium exchange method of measuring A, particularly for sheet cellulose samples.

The results for amylose and amylopectin differ from those of Ceh and Hadzi (12), and suggest the interesting possibility that starch can exist in stable forms which are either completely or only partially accessible. The xylan from birch wood is a linear polysaccharide like cellulose and similarly was only partially accessible. The differences between commercial wood fibers were small, suggesting that pulping processes have little effect upon the properties of cellulose.

It was surprising that A^0 did not increase to a greater extent with increasing relative humidity since reactive hydroxyl groups are believed to be formed as a result of disrupting hydrogen bonds by water (15). However, X-ray diffraction studies have indicated that the fraction of crystalline material is the same in wet and dry cellulose (16), although recently it was reported that a small reduction of the molecular order in crystalline regions occurred with increasing relative humidity (17). The similarity of the effect of relative humidity upon the crystalline and inaccessible portion supports the concept of stable, discrete crystallites which largely do not participate in the swelling or in many chemical reactions of cellulose. There is evidence, however, that crystallites tend to migrate in the structure during changes of relative humidity (2) as described further in the following paper (8).

The most interesting results were the apparently anomalous properties of amylose and birch xylan which implied that accessibility was not necessarily dependent upon the crystallinity. It is likely that steric effects dominate the extent of exchange of hydrogen in cellulose as found with other reactions (18), and that the conditions which preclude exchange can exist independently of crystallinity. The extent of exchange may therefore be expected to vary in different crystals.

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HYDROGEN EXCHANGE BETWEEN CELLULOSE AND WATER

II. INTERCONVERSION OF ACCESSIBLE AND INACCESSIBLE REGIONS[1]

O. SEPALL[2] AND S. G. MASON

ABSTRACT

Exchange of hydrogen between water and some of the inaccessible hydroxyl groups in cellulose resulted from changes in the relative humidity and was most pronounced near 0 and 100% RH. A similar exchange occurred during prolonged immersion in liquid water. The extent of the reaction varied in different samples, increased with temperature, and was reproducible. By repeatedly wetting and drying cellophane, the exchange was cumulative and all the hydroxyl groups became exchanged after up to 100 cycles. The accessibility decreased slightly after repeated drying. The measurements were made by exchange with deuterated and tritiated water.

The behavior was interpreted as an interchange of ordered and disordered regions resulting from molecular rearrangement caused by interaction between cellulose and water.

INTRODUCTION

When cellulose is dried from heavy water (D_2O) (1) or tritiated water (HTO) (2), some of the hydrogen isotope becomes incorporated in inaccessible hydroxyl groups, i.e., hydroxyls which will not exchange hydrogen when the cellulose is re-exposed to water. Mann and Marrinan (1) suggested that the phenomenon was partly due to a reduction in the number of reactive hydroxyls by drying. However, Lang and Mason (2) showed by repeatedly wetting and drying cellophane in HTO that inaccessible hydroxyl groups were progressively exchanged with little change in the accessibility. The theory was proposed that during wetting and drying, a rearrangement of molecules occurs in cellulose and results in a partial interchange of accessible and inaccessible regions (2).

The present investigation was undertaken to study the causes of the changes in molecular order. It was also of interest to determine if all crystallites could be disrupted by repeatedly wetting and drying cellulose a sufficient number of times as appeared likely from the previous studies (2). The work included a study of the influence of temperature, relative humidity, and the number of cycles upon the irreversible exchange during drying.

The quantities measured were similar to those investigated by Lang and Mason (2) and are defined as follows. The accessibility A_n is the extent of the exchange reaction with water at 75% relative humidity (RH) and 25° C after n drying cycles. The quantity $\delta\omega_n$ represents the inaccessible hydroxyl groups reacted with water during the nth cycle while W_n is the net inaccessible hydroxyl groups reacted during n cycles. The three quantities were expressed as percentage of the total hydroxyls. Measurements were made by both tritium and deuterium exchange.

EXPERIMENTAL PART

Methods

1. Measurement of Accessibility

The accessibility (A_n) was determined by the HTO exchange method described previously (3). The measurement was calibrated for each material by determining the exchange of deuterium with D_2O by the gravimetric method (3); this was done to eliminate

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Contribution from the Physical Chemistry Division, Pulp and Paper Research Institute of Canada, and the Department of Chemistry, McGill University, Montreal, Que.
[2]*Holder of a Canadian Industries Limited Fellowship, 1960–61.*

the small error resulting from the effect of surface roughness upon the measurement of tritium radioactivity (3). The results were averages for two determinations and were reproducible to $\pm 1\%$.

Since it was known (3) that A_n varies slightly with relative humidity (RH), standard conditions for the measurement were arbitrarily selected as 75% RH and 25° C.

2. Drying Cycles Using HTO

Three types of cycles, designated as D_1, D_2, and D_3 and representing regions of intermediate, low, and high RH respectively, were employed to study limited ranges of RH. Shorter drying times than required for equilibrium conditions (e.g., 23 hours to achieve moisture equilibrium in cellophane (4)) were used to permit the study of the effects of large numbers of cycles within a reasonable time.

The procedure for each type of cycle is outlined below.

Cycle D_1 (partial drying).—The treatment consisted of exposing the sample to HTO vapor during a cyclic change of RH and was conducted in a special counter designed for use of tritiated sheets (5). Only single cycles were studied. The initial exposure was to H_2O followed by HTO at the same RH. The vapor pressure of the HTO was then altered by changing the temperature of the HTO liquid reservoir. The duration of each exposure was 4 hours.

Cycle D_2 (rigorous drying).—The cycle consisted of exposing the sample, as in D_1, to HTO vapor and then vacuum-drying for 15 hours at 1 μ Hg and various temperatures. The exposure to HTO was usually for 8 hours at 75% RH and 25° C. For repeated treatments, one cycle, including the determination of tritium in the sample after drying, was performed each day.

Cycle D_3 (immersion and partial drying).—The sample was immersed in HTO liquid and partially dried at different temperatures kept constant during the experiment. The procedure devised by Lang and Mason (2) was used. The sample and a quantity of HTO liquid (30:1), large enough so that dilution effects could be neglected, were sealed in an evacuated, inverted U tube with the sample held at one end. During drying, the HTO arm was placed in an ice-water bath and the other in a water thermostat at the desired temperature. The wetting step was performed by tilting the U tube and allowing the HTO to flow to the sample end. Two hours were usually allowed for each step; preliminary experiments with cellophane showed that the exchange was over 80% completed during this period. For repeated treatments, three cycles were performed each day; one wetting step (conducted overnight) took 14 hours.

3. Reacted Inaccessible Hydroxyls by HTO Exchange

During each of the drying cycles, tritium became incorporated in both inaccessible and accessible hydroxyl groups. For example, after n successive D_2 cycles, the total tritium activity corresponded to (A_n+W_n). By exposing the sample to H_2O and thus removing the labile tritium (A_n), W_n alone was determined by the following procedure. The treated sample was placed in a 2-cm glass tube through which air at 75% RH and 25° C was circulated for 8 hours; 16 hours gave the same result. The residual tritium activity in the sample was measured in a counter (5) identical with that used for accessibility measurements but without the accessories for conducting the exchange reaction. The fraction of tritiated hydroxyl groups (W_n), corrected for isotope effects, was calculated from the measured counting rate as previously described for A_n (3).

It would have been desirable to end all drying cycles at 75% RH to avoid further change of sample moisture content during removal of labile tritium. Although this was done only in certain cases, the cycles were always terminated at a RH $< 95\%$ and, as will be shown later, the difference in RH during the final drying had little effect.

For the measurement of $\delta\omega_n$ a similar procedure was used. The $(n-1)$ cycles were performed in H_2O and the nth cycle in HTO. The labile tritium was removed as described above and the residual tritium activity corresponded to $\delta\omega_n$. It should be noted that, by definition, $\delta\omega_1 = W_1$.

The results were usually reproducible to $\pm 5\%$ or less. In cellophane, however, differences were as large as 50% and may have been due to a lack of uniformity in the properties as a result of manufacturing conditions.

The quantities $\delta\omega_n$ and W_n defined by Lang and Mason (2) were determined after immersing the sample in H_2O liquid and air-drying, and are therefore lower than the present values because, as will be shown, a large fraction of the inaccessible hydroxyl is reacted during drying.

4. Reacted Inaccessible Hydroxyls by D_2O Exchange

To corroborate the experiments using HTO, a method of measuring (A_n+W_n) for cycle D_3 was devised based on the gravimetric measurement of A using D_2O (3). The method has the advantage that the samples are not required in sheet form. The apparatus is shown in Fig. 1 and served the same purpose as the U tube described above in the

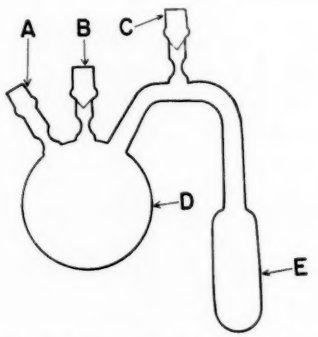

FIG. 1. Cell for measuring A_n+W_n by D_2O exchange. A, outlet; B and C, outlet with break-seals; D, 50-ml glass flask; E, 20-ml receiver.

corresponding experiment with HTO. It consisted of a 50-ml glass flask (D) connected by an inverted U tube to the 20-ml vessel (E). The three outlets (A, B, and C) were of suitable diameter to fit a Veeco Quick Vacuum Coupling on a vacuum manifold and B and C contained break-seals. The expanded sections in the outlets helped to form the vacuum seal created by an O-ring. The vessel could be sealed by fusing at the constrictions.

To correct for air buoyancy during weighings, the volume of the apparatus was determined by weighing the flask empty (M_0), and filled with water. A 3-g sample of cellulose was placed in the flask and dried rigorously by evacuating through outlet A for 48 hours at 75° C and 1 μ Hg (6). The flask was sealed by fusing at the constriction and weighed (M_1). The break-seal in B was opened by thrusting a steel rod in the outlet. To reduce subsequent dilution effects, the sample was exchanged with pure (99.7%) D_2O vapor by circulating a mixture of D_2O and air, at 75% RH, through the vessel for 4 days. The procedure resulted in a limiting exchange corresponding to the accessibility A_0*. A quantity (15.0 ml) of D_2O liquid was then added to the flask, which was sealed after evacuation,

**The limiting or equilibrium accessibility was previously (3) denoted by A^0. In this paper it is more convenient to designate this quantity as A_0 since it corresponds to A_n when $n = 0$.*

with the flask immersed in liquid air to prevent loss of D_2O. The sample was subjected to the desired number of D_3 cycles as in the corresponding HTO experiment. The break-seal in outlet C was opened and the flask evacuated to dry the sample, then sealed under vacuum, and weighed (M_2).

The increase of mass ΔM, corresponding to exchange of H atoms on hydroxyl groups by D atoms, was determined from the difference of the weight (M_2-M_1) corrected for changes in air buoyancy. The mass M of the sample was determined similarly from (M_1-M_0).

To determine (A_n+W_n) it was necessary to correct for the dilution of D_2O resulting from exchange with ($A_n+W_n-A_0$) of the hydroxyl groups which did not react during the exposure to D_2O vapor. For a sample of mass M the dilution effect corresponded to ($0.167W_nM$) g H_2O; it was reasonable to assume that ($A_n+W_n-A_0$) = W_n because the correction was small and, as will be shown, $A_n = A_0$ (approx.). The final purity P of D_2O was therefore given by

$$P = 99.7 - \frac{100(0.167W_nM)}{(15.0\times.997)+(0.185A_0M)}.$$

In this relation, 99.7% is the initial purity; the second term represents the percentage dilution by H_2O.

The quantity (A_n+W_n) was then calculated from the relation

$$A_n+W_n = \frac{100}{P} \times \frac{100(\Delta M+\Delta M_B)}{0.0185M}$$

where 0.0185 represents the fractional increase in mass for cellulose after all hydroxyl groups have been exchanged by deuterium and ΔM_B is the change in mass in the same experiment but conducted in H_2O. ΔM_B was a correction for a loss in mass probably due to degradation of the cellulose.

The value of (A_n+W_n) was estimated first by assuming no dilution of D_2O (P = 99.7%). P was then calculated and a second approximation of (A_n+W_n) gave the desired accuracy.

Materials

The samples listed below were used.

1. Cellophane (supplied by Dupont of Canada Limited) was air-dried viscose film containing no additives.

2. Wood cellulose was a softwood acetate grade woodpulp with 96% alpha cellulose (TAPPI method).

3. Cotton linters were dewaxed by alcohol–benzene extraction.

4. Bacterial cellulose was kindly supplied by Dr. R. J. Colvin of the National Research Council, Ottawa.

5. Amylose was the commercial grade supplied by Stein Hall and Co. The samples were prepared as before (3).

RESULTS

Comparison of Cycles

Various modifications of the three drying cycles were tried to establish the conditions under which the interconversion of accessible and inaccessible hydroxyls occurred most readily. The experimental conditions and results for wood cellulose are listed in Table I;

TABLE I

W_1 for various drying cycles for wood cellulose

Expt. No.	Cycle	Temp. (°C)	Conditions	W_1 (%)
1	Control	30	(1) Conditioned in H_2O, 8 hr at 65% RH (2) Exposed to HTO, 8 hr at 65% RH (3) Exposed to H_2O, 8 hr at 65% RH (as for measurement of W_1)	0.6
2	Control	100	(1) Conditioned in liquid H_2O, 4 hr (2) Immersed in liquid HTO, 4 hr (3) Immersed in liquid H_2O, 8 hr (4) Air-dried	0.9
3	D_1	30	(1) Conditioned in H_2O, 4 hr at 60% RH (2) Exposed to HTO, 4 hr at 60% RH (3) RH changed to 14% for 2 hr (4) RH returned to 50% for 2 hr (5) Exposed to H_2O, 8 hr at 60% RH (as for measurement of W_1)	1.7
4	D_1	30	(1) Same as No. 3 except in step (3) RH changed to 90% for 2 hr	1.
5	D_2	25	(1) Exposed to HTO, 4 hr at 75% RH (2) Dried 15 hr at 1 μ Hg (3) Exposed to H_2O, 8 hr at 75% RH (as for measurement of W_1)	2.
6	D_2	25	(1) Dried 15 hr at 1 μ Hg (2) Exposed to HTO, 8 hr at 75% RH (3) Exposed to H_2O, 8 hr at 75% RH (as for measurement of W_1)	2.7
7	D_2	See conditions	(1) Dried 15 hr at 1 μ Hg, 75° C (2) Exposed to HTO, 8 hr at 75% RH, 25° C (3) Exposed to H_2O, 8 hr at 75% RH, 25° C, as for measurement of W_1	4.1
8	D_3	25	(1) Immersed in liquid HTO, 2 hr (2) Dried 2 hr at 20% RH (3) Exposed to H_2O, 8 hr at 75% RH (as for measurement of W_1)	5.0

two control experiments in which the sample was exposed to HTO and subsequently to H_2O without changing the RH are included.

In both control experiments (Nos. 1 and 2), W_1 was small but significant. Experiment 2 was similar to one described by Mann and Marrinan (1), who found that cellophane retained small quantities of deuterium after immersion in liquid D_2O followed by H_2O. The same experiment performed by Lang and Mason (2) in HTO liquid at 25° C gave values of 0 and 0.2% for W_1.

In expts. 3 and 4, cyclic changes of RH in a range from 15 to 95% yielded larger values of W_1 than expt. 1 although the time of exposure to HTO vapor was the same.

In expt. 5 (cycle D_2) the sample was exposed to HTO vapor only during desorption and in 6, only during adsorption; the values of W_1 were larger than in Nos. 3 and 4, presumably because of the lower RH during drying, and were approximately equal, showing that the change occurred with both increasing and decreasing moisture content.

Experiment 7 (cycle D_2) was similar to No. 6 with the exception that the sample was dried at 75° C to remove strongly bound moisture (6). A pronounced increase in W_1 occurred which was undoubtedly associated with adsorption of HTO at zero regain. The corresponding desorption experiment, in which the sample was dried from HTO to zero regain at 25° C, could not be studied because all the sorbed water could not be removed at this temperature under the conditions of evacuation used.

The greatest W_1 was formed in No. 8 in which the sample was immersed in HTO liquid and dried in contact with HTO vapor at 20% RH; the large effect apparently occurred in the range of relative humidity from 95 to 100%.

It was concluded from the experiments that (*a*) tritiated inaccessible hydroxyls were formed on changing the RH up or down and (*b*) the effect was most pronounced at the

extreme ends of the drying cycle, i.e., at 0 and 100% RH. As found earlier (1, 2), a smaller but significant amount of reacted inaccessible cellulose resulted from exposure at constant RH.

The influence of other variables was studied with cycles D_2 and D_3 because they incorporated the two extreme conditions which produced the greatest changes.

Effect of Temperature

The influence of temperature upon W_1 in wood cellulose and cellophane was investigated between 25 and 100° C for cycles D_2 and D_3. The specified temperature for cycle D_2 was for the vacuum-drying only, whereas in cycle D_3, it was for both immersion and partial drying.

The results (Fig. 2) show that W_1 increased with temperature in both cycles.

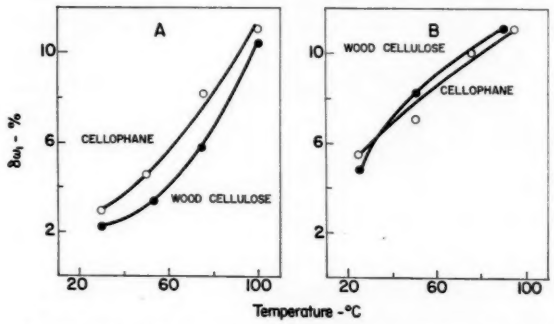

FIG. 2. Effect of temperature upon W_1. A, cycle D_1; B, cycle D_3.

Apart from the temperature during drying, two secondary factors may have contributed to the increase of W_1 with temperature, namely, heating the sample from 25° C to the higher temperature in cycle D_2 and the lower RH when drying at higher temperature in cycle D_3. To determine if the temperature of drying was of importance, the modified cycles described in Table II were conducted on wood cellulose.

TABLE II

W_1 for modifications of cycles D_2 and D_3 on wood cellulose

Expt. No.	Cycle	Conditions	W_1* (%)
9	D_2	(1) Exposed to HTO, 8 hr at 75% RH, 25° C (2) Dried 15 hr at 1 μ Hg, 75° C	5.8
10	D_2	(1) Exposed to HTO, 2 hr at 2.3% RH, 75° C (2) Dried 15 hr at 1 μ Hg, 75° C	6.6
11	D_2	As for expt. 9 with drying at 100° C	12.1
12	D_2	As for expt. 9 with drying at 100° C for 40 hr	11.7
13	D_3	(1) Immersed in liquid HTO, 2 hr, 50° C (2) Dried 12 hr at 95% RH, 50° C	8.3
14	D_3	As for expt. 13 with drying for 2 hr at 15% RH	8.5
15	D_3	As for expt. 13 with drying for 2 hr at 1 μ Hg	8.5

*In each experiment, W_1 was measured after the sample was exposed to H_2O for 8 hours at 75% RH and 25° C, as described in the text.

Cycle D_2 at 75° C, as described above, is presented for comparison as expt. 9. In expt. 10 (cycle D_2), the temperature (75° C) was constant throughout and the RH was

lower (2.3%) during exposure to HTO. The comparable values for W_1 in Nos. 9 and 10 show that the temperature during drying at low RH determined the value of W_1 in cycle D_2. Continued heating of the dried sample produced no additional change (expts. 11 and 12).

In expts. 13 to 15 (cycle D_3) the RH during drying ranged from 10^{-3} to 95% and similar values of W_1 were found. Therefore the temperature during soaking, or drying, from 100 to 95% RH determined the magnitude of W_1 in cycle D_3.

Comparison of Samples

Values of W_1 for cycles D_2 and D_3 are listed in Table III for different types of cellulose

TABLE III

W_1 for different samples

Sample	A_0* (%)	W_1 (%)	
		Cycle D_2 at 75° C	Cycle D_3 at 50° C
Cellophane	79	8.3	7.1
Wood cellulose	58	5.8	8.5
Cotton linters	40	4.3	9.1
Bacterial cellulose	42	2.6	8.5
Amylose	98	<0.1	<0.1

*From ref. 3.

and amylose. With cycle D_2, W_1 for cellulose increased with increasing A_0 whereas with cycle D_3 there appeared to be no correlation. With amylose, $W_1 = 0$ in both cycles as would be expected if the substance were completely accessible. Although the measured accessibility of amylose was consistently a little lower than 100% the difference probably did not indicate the presence of unreactive hydroxyls but an impurity in the sample, as discussed previously (3).

Repeated Cycles (HTO)

(a) Effect upon A_n and $\delta\omega_n$

Table IV lists values of A_n and $\delta\omega_n$ for samples which have undergone $(n-1)$ drying cycles (D_2 and D_3) in H_2O.

TABLE IV

Effect of repeated drying upon A_n and $\delta\omega_{n+1}$ by HTO exchange

Sample	Drying history*			A_n (%)	$\delta\omega_{n+1}$	
	Cycle	n	Temp. (°C)		Cycle D_2 at 75° C	Cycle D_3 at 50° C
Wood cellulose		0		58	5.8	8.5
,,	D_2	2	100	58	6.4	
,,	D_2	10	75	57	6.5	7.8
,,	D_3	10	95	56	6.0	7.5
,,	D_3	50	50	55	6.2	7.0
Cellophane		0		79	8.3	7.1
,,	D_2	10	75	76	10.7	6.8
,,	D_3	50	50	76	9.5	4.9
Bacterial cellulose		0		42		
,,	D_2	8	75	41		

*Before the present experiments, cellophane and bacterial cellulose had been air-dried while wood cellulose had probably been dried at >100° C during manufacturing.

The accessibility was decreased slightly by drying, e.g., from 58 to 55% for wood cellulose after 50 D_3 cycles at 50° C, but the change in A_n during the first few cycles was negligible. Values of $\delta\omega_n$ for cycle D_2 increased with n, while for cycle D_3 the value decreased. The greatest change (from $\delta\omega_1 = 7.1\%$ to $\delta\omega_{51} = 4.9\%$ after 50 D_3 cycles at 50° C) occurred in cellophane and was similar to the earlier result (2).

(b) Effect upon W_n

The previously reported (2) increase of W_n with n was investigated under various conditions to determine if exchange with all hydroxyl groups, corresponding to $(A_n+W_n) = 100\%$, would occur when n was made large. Figures 3 and 4 show the results for cycles

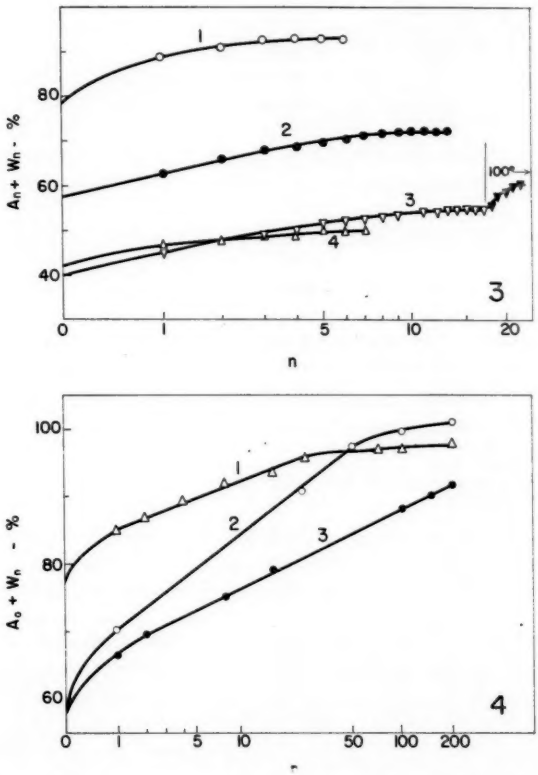

FIG. 3. A_n+W_n for cycle D_2 at 75° C. Curve 1, cellophane; 2, wood cellulose; 3, cotton linters; 4, bacterial cellulose.

FIG. 4. A_0+W_n for cycle D_3. Curve 1, cellophane at 50° C; 2, wood cellulose at 100° C; 3, wood cellulose at 50° C.

D_2 and D_3. In the latter experiments W_n was determined, and the data are shown as (A_0+W_n); this procedure is reasonable since A_0 and A_n are approximately equal. In both figures, log $(n+1)$ is plotted to include results for large n's while still showing the large changes occurring in the early cycles.

For cycle D_2 at 75° C, (A_n+W_n) was found to increase to a characteristic limiting value (Fig. 3) which remained constant to $\pm 0.1\%$ after several further cycles. By

increasing the drying temperature, as shown with cotton linters, a higher value was obtained. However, complete exchange was not achieved and higher temperatures than 100° C could not be studied with the present apparatus.

Using cycle D_3, (A_0+W_n) for wood cellulose and cellophane increased linearly with log $(n+1)$ until approximately 100% and then leveled off, indicating that complete exchange had occurred. The further increase in (A_0+W_n) above 100% was undoubtedly due to the continued increase of W_n as A_n decreased slowly with n (see Table IV). The quantity (A_n+W_n) would have been expected to remain constant at 100%.

The rate of increase of (A_0+W_n) with n was greater at higher temperature; it appeared that more than 20 times as many cycles would have been required at 50° C than at 100° C to reach $(A_0+W_n) = 100\%$.

(c) Effect of Prolonged Soaking in HTO upon W_n

Each of the D_3 cycles involved a period of immersion in liquid HTO. At large n the total immersion period, although not continuous, became large and, on the basis of the control experiment (No. 2, Table I), may have accounted for some of the increase in W_n reported above. The magnitude of the effect was estimated by measuring the variation of W_1 for cycle D_3 using soaking times from 2 hours to 135 days and the usual drying time of 2 hours.

Results for wood cellulose are given in Table V for temperatures from 25 to 100° C.

TABLE V

Effect of prolonged soaking upon W_1 in cycle D_3 for wood cellulose

Soaking time	Temperature (°C)	W_1 (%)
2 hours	25	5.0
64 days	,,	8.9
135 days	,,	10.2
2 hours	50	8.5
64 days	,,	16.4
135 days	,,	17.8
2 hours	100	12.2
15 days	,,	26.6
64 days	,,	28.9

The value of W_1 increased with soaking time and temperature. For large numbers of D_3 cycles, the exchange due to soaking undoubtedly constituted a large part of W_n. For example, the increase in W_1 due to the first 15 days of soaking at 100° C was 14.4% (Table V) while $(W_{50}-W_1)$ for 49 D_3 cycles, which included a total of 17 days' soaking, was 27.2%. The experiments with D_3 cycles thus measured the combined effects of repeated drying and of soaking.

Repeated Cycles (D_2O)

Two values of (A_n+W_n) were measured for cycle D_3 using D_2O with cellophane and wood cellulose to check the above results with HTO.

The results listed in Table VI are in satisfactory agreement with cellophane but not with wood cellulose. The error is believed to be in the measurement of deuterium exchange and in the correction term ΔW_B (see Experimental Part) which represents the loss in mass of cellulose probably due to chemical degradation. Since the degradation could be estimated only in H_2O and would be expected to be different in D_2O, the correction was

TABLE VI

Comparison of results for cycle D_3 by exchange with D_2O and HTO

Sample	n	ΔM (%)	ΔM_B (%)	(A_n+W_n) (%), D_2O exchange	(A_0+W_n) (%), HTO exchange
Cellophane	50	1.57	−0.15	94	97
Wood cellulose	185	1.09	−0.31	76	90

probably invalid. Evidence of chemical reaction in cellulose after the cyclic drying was the distinct yellowing of the sample which had occurred, particularly where it was in contact with glass.

The gravimetric measurement of deuterium exchange with D_2O has, however, been used with excellent results for the measurement of accessibility (3). It appears that the degradative processes in cellulose have a significant effect upon the mass of cellulose only at high temperature (3) and during prolonged exposure to water.

DISCUSSION

The results may be summarized as follows:

1. exchange with the inaccessible hydroxyl groups in cellulose accompanied a change of relative humidity, either up or down;
2. the effect was most pronounced near 0 and 100% RH and increased with temperature;
3. the magnitude of the exchange was characteristic for different forms of cellulose;
4. when cellulose was wetted and dried repeatedly, the exchange increased until, as previously predicted, all hydroxyl groups had reacted;
5. the accessibility A of cellulose decreased slowly with large numbers of drying cycles; and
6. reaction with inaccessible hydroxyls also occurred during prolonged soaking and increased with temperature.

The formation of reacted inaccessible hydroxyls is assumed to result from the conversion of accessible regions to the inaccessible form, and thus implies localized changes in the degrees of molecular order. According to more recent concepts (7), the fine structure of cellulose can be described by a distribution of lateral order, the highly ordered regions corresponding to crystallites and the low-ordered, representing amorphous cellulose. Some evidence suggests that the inaccessible regions are highly ordered cellulose such as the interiors of crystallites, and perhaps also strongly hydrogen-bonded amorphous regions. For example, samples are graded in the same order by measurements of both accessibility and amorphous fraction by X-ray diffraction (7). Furthermore, the inaccessible hydroxyls in cellulose and crystalline cellopentaose give similar infrared absorption bands (1). It was also found that the change in accessibility with relative humidity is small (3) and similar to the effect upon the amorphous fraction determined by X-ray diffraction (8, 9). However, recent studies suggest that the accessibility of regenerated cellulose and of single crystals (10) of cellulose II may be similar (11). Anomalous behavior is also shown by substances other than cellulose. For example, a partially crystalline amylose was completely accessible while an amorphous xylan was only 52% accessible (3). It is evident, therefore, that one cannot unequivocally assume that inaccessible cellulose corresponds to the crystalline part although the two properties appear to

be related in some way. In the present discussion it will be assumed for simplicity that the disruption of inaccessible regions represents destruction of crystallites.

To explain the accessible–inaccessible transformations during drying it was suggested previously that a molecular rearrangement occurred at an intermediate moisture content favorable for the formation of hydrogen bonds and for mobility of molecular chains (2). Under these conditions a dynamic equilibrium was believed to exist between accessible and inaccessible cellulose. It was assumed that at lower moisture content the cellulose chains became immobilized by interchain hydrogen bonding, while the swelling in the wet state prevented the formation of hydrogen bonds between cellulose molecules. Since the present results show that transformations occurred in cellulose over a wide range of moisture contents corresponding to relative humidities from 0 to 100%, this hypothesis does not appear to be valid. Furthermore, the changes were greatest at very low and high relative humidity when conditions were least favorable for the proposed equilibrium. The slight decrease of accessibility with decreasing relative humidity (3) may partly account for the formation of inaccessible cellulose during drying. However, the same effects were observed during sorption of water and have not been fully explained.

It is now proposed that molecular rearrangements, which have previously been assumed to occur only in the amorphous regions, with changes in the quantity of sorbed water (12), are also responsible for disrupting and reforming ordered regions. The destruction of the crystallite can be attributed to molecular stresses created in amorphous regions by the swelling action of sorbed water or by hydrogen bonds tending to draw cellulose molecules together when water is removed. Since the molecules extend through many regions of different order, the stresses, which on a molecular scale must become exceedingly high, are transmitted throughout the structure. The pronounced changes occurring in W_1 near 0 and 100% RH can be associated with characteristic interactions of cellulose and water at these relative humidities. At zero regain, water is very strongly bonded to cellulose, and Urquhart (13) has suggested that each H_2O molecule is bound to two hydroxyl groups. The resultant linking by the sorbed water can be conceived as drawing cellulose molecules together and causing a rearrangement. At the other extreme, the pronounced swelling near 100% RH could have the same result. The increasing transformation with temperature may be attributed to greater mobility of cellulose molecules; the reduction of sorption hysteresis with temperature has been ascribed to the same effect (14).

With the present rudimentary concepts it is not possible to consider the significance of the magnitude of the changes under various conditions. Presumably the results reflect the interplay between the network geometry of the molecular chains and the drying treatment. It is possible that the magnitude of these effects may characterize the fine structure and indicate, for example, the ease of molecular rearrangements in different samples.

The disruption of crystallites by molecular stresses suggests that similar effects may arise by mechanical deformation. It is known that by stretching cellulose, an orientation is created in the structure. However, little change in crystallinity was noted; cellulose acetate filaments, however, stretched up to 20 times their original length and when saponified underwent an increase in crystallinity (15). Whether crystallites were disrupted during the stressing was not known. With wool fibers, stretching was found to increase the reactivity of the disulphide bonds and was interpreted as a configurational change influencing the extent of the reaction (16). This effect appears to be somewhat analogous to the exchange with inaccessible hydroxyl groups in cellulose during drying.

The small effect of drying upon the accessibility and upon the values of $\delta\omega_n$ suggests that there is little permanent change in the cellulose. Other investigators have similarly reported only small changes in cellulose reactivity to nitration (17), hydrolysis (18), and exchange with D_2O (19) by repeated wetting and drying. The effects are not always the same; for example, accessibility to iodine absorption increased after drying (20) while accessibility to thallation decreased (19). It is therefore not clear whether the degree of molecular order is affected by drying, but in any event the change cannot be large. The reduction of accessibility after large numbers of drying cycles in the present work indicated an increase of the crystalline fraction. Recrystallization may have resulted from hydrolysis, since the DP of cellulose has been found to decrease in similar experiments (21).

A result of interest was the increasing exchange in the inaccessible regions with higher temperature during prolonged immersion in water. It appeared that at some temperature greater than 100° C, the cellulose may be completely accessible for the usual reaction time of several hours allowed in the measurement. The result suggests that crystallites may be continuously being disrupted and reformed by the thermal energies of the molecules. The effect may be greater in the presence of liquid water because, like other plasticizers (22), it would be expected to lower the second-order transition temperature and thereby permit the molecular rearrangement to occur more readily.

The transient existence of crystallites, as suggested by the present results, would also be expected to play a role in other chemical and physical changes in cellulose.

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OZONOLYSIS OF UNSATURATED FATTY ACIDS

I. OZONOLYSIS OF OLEIC ACID[1]

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ABSTRACT

Ozonolysis of oleic acid in methanol, a reacting solvent, with subsequent decomposition of the ozonide products by hydrogen peroxide in formic acid, gives yields of principal dicarboxylic acid fission products exceeding 95% with a minimum of secondary acidic products. The method is highly reproducible and offers unique advantages in the total recovery of the dicarboxylic acids and the elimination of peroxidic materials. The principal non-acidic by-products were tentatively identified as the C_8 alcohols and their formyl esters.

INTRODUCTION

The study of the position of the ethylenic double bonds in long-chain fatty acids is important in connection with the isolation of various naturally occurring fatty acids, and even more important in fields where the chemical treatment of the fatty acid by partial hydrogenation, alkali fusion, or other prototropic reactions leads to the formation of positional isomers. In the former instance, any reasonable yield of principal fission products resulting from the scission of the double bond is satisfactory for bond location, particularly as naturally occurring fatty acids are usually homogeneous in bond position and impurities will differ enough structurally to be recognized as such. In the case of mixtures of positional isomers, the over-all yield is less important than two other factors which must be considered. Firstly, the occurrence of abnormal fission products such as lower homologues of the principal fission products must be reduced to a minimum, and secondly, the method of recovery and analysis of the products must be such that the analysis reflects the true proportions of these products.

Scission of the double bonds is usually achieved by oxidative fission. Some work has been done to give aldehydes as primary products (1–9), but the more extensive application of this technique has always been in the direction of obtaining the more tractable acidic fission products. Ozonolysis is an attractive technique owing to the complete reaction of ozone with the starting material (8) and the simplicity of the reagents and procedures generally adopted. The chief disadvantages, other than the necessity of an ozone generator, have always been the nominal yields (70–85%) (10–16) of primary acidic fission products, the associated formation of carbonyl, peroxidic, and polymeric materials (1, 7, 17), and the presence of one or more secondary acidic oxidation products. The latter can cause considerable uncertainty where minor amounts of positional isomers are being determined, while by-products hinder both separation and analysis of the products (10, 18). Certain procedures do give high yields of products, but the silver oxide oxidation method of Asinger (3, 12, 19) and the procedure of Klenk and Bongard (20) are somewhat complex, while that of Pryde *et al.* (8) yields aldehydes as principal products.

The most common alternatively employed oxidizing agent, potassium permanganate, also gives only moderate yields of acidic products and considerable secondary oxidation (11, 12, 18, 21–24). The recently introduced permanganate-catalyzed periodate method of von Rudloff (3, 25–28) is much more attractive owing to the simplicity of the method

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and the very high yields of primary products. These techniques do suffer from a disadvantage in that recovery of the primary products, in particular the dicarboxylic acids, must be made by extraction from a highly mineralized aqueous solution, with consequent risk of losses of the more soluble lower dicarboxylic acids. In the past this factor has led to erroneous results in determining the relative proportions of positionally isomeric double bonds (13, 24, 29, 30).

If ozonolysis is to be considered as a useful method for the sensitive determination of double-bond position in long-chain fatty acids it must satisfy the following requirements:

1. the method should have a high yield of primary products and should give rise to the least possible amounts and variety of "abnormal" products;
2. the procedure of working-up should give the highest possible recoveries of related products even when these have differing physical properties;
3. the method should be simple and reproducible;
4. the method of analysis should be sensitive and accurate.

It was deemed desirable that the principal products should be acidic in order to capitalize on the stability and low volatility of the desirable dicarboxylic acids. The method outlined below, based on ozonolysis in methanol, a reacting solvent, with decomposition of the ozonide product with hydrogen peroxide in formic acid, satisfies the above requirements. The yield of principal dicarboxylic acid product averages 95%, accompanied by only 2–4% lower homologous dicarboxylic acids and 2–4% other materials. The principal non-dicarboxylic acid by-product has been tentatively identified as the alcohol formed from the principal acidic product through the loss of one carbon atom, occurring both as the alcohol and as its formyl ester. Since only volatile solvents and reagents are employed, and the monocarboxylic acids are removed by steam distillation, total recovery of the dicarboxylic acids present is possible. The method is relatively simple and while some variation is found in the proportion of non-dicarboxylic products to dicarboxylic acids, the ratio of total secondary dicarboxylic acid products to principal dicarboxylic acid product is remarkably stable. The mixture of products is readily analyzed with a high degree of accuracy by gas–liquid chromatography. The methanol – formic acid procedure is derived from that employed by Bailey (31, 32) for the production of adipic acid by ozonolysis of cyclohexene, by Perry (33) in the ozonolysis of norbornylene, and by Pryde *et al.* (8) in the ozonolysis of methyl oleate to give carbonyl products. Methanol has also been employed recently by other workers in the ozonolysis field (34–37), although not necessarily in exploitation of the same type of reaction.

EXPERIMENTAL

Pure oleic acid was prepared from filbert oil (38) via the methyl esters by established procedures (39, 40). The following properties were determined: iodine value 91.2 (Wijs), 89.1 (by precision semimicro hydrogenation (41)) (calc. 89.9); n_D^{25} 1.45785 (cf. n_D^{25} 1.45792 (42)). Ultraviolet spectrophotometry showed no diene or triene content prior to alkali isomerization* and the latter procedure showed a diene content of 0.1% or less. Gas–liquid chromatography of the methyl ester showed only an oleate peak.

It has been reported that an oleic acid prepared from olive oil by standard methods contained appreciable amounts of positional isomers (29). The present and other studies (13, 14) have confirmed that oleic acid homogeneous in bond position can be readily

**Modification of the method of Vandenheuvel and Richardson (43); 21% KOH in ethylene glycol, effective heating time 15 minutes.*

prepared. The ultimate criterion of purity in all cases must be the analysis of both mono- and di-carboxylic acid fission products. Thus the presence of small amounts of suberic acid in the dicarboxylic acid product does not signify the presence of octadec-8-enoic acid unless decanoic acid can be found in the complementary monocarboxylic acids. In the present studies the occurrence of both mono- and di-carboxylic acid products containing 10 carbon atoms has been noted in amounts of $\leqslant 0.1\%$. Thus the amounts of octadec-8- and octadec-10-enoic acids cannot have exceeded this level.

Ozone was furnished by a pyrex corona discharge tube (4). At an oxygen flow rate of 1.1 l./hr, the ozone content was determined as 2.2% (w/w) by decomposition of potassium iodide (44). A dry-ice trap supplemented chemical drying of the oxygen.

1. Ozonolysis in Methanol

Pure oleic acid (ca. 1 g) was weighed in a long-necked, 100-ml standard taper flask. Absolute methanol (Fisher Spectrophotometric Grade, 40 ml) was added and the solution was chilled to $-30°$ C and treated with ozone-containing oxygen until complete absorption ceased. The flask was fitted with a distilling head in order to admit a capillary tube (nitrogen) and was connected through a dry-ice trap to a mechanical vacuum pump system. The flask was immersed in water at room temperature and vacuum applied in stages until all the methanol was removed and the pressure was less than 1 mm. Formic acid (98%, 25 ml) was added to dissolve the syrupy residue and hydrogen peroxide (30 or 50% aqueous solution, 10 mole-equivalents) added. A reflux condenser was immediately fitted to the flask and gentle warming induced an exothermic reaction, on completion of which the mixture was heated for 1 hour on a steam bath. The peroxide and peracid content fell rapidly below measurable limits.

Water (20 ml) was added and the solution transferred to a continuous steam-distillation unit, a few drops of acetone serving as rinse. In this case, 200 ml of distillate was collected for recovery of the nonanoic and associated monocarboxylic acid products. The dicarboxylic acid solution was transferred to a distillation unit and evaporated nearly to dryness under aspirator vacuum. Toluene was added in small lots (ca. 10 ml) and removed under reduced pressure until the distillate was free of water and formic acid (45). Further vacuum treatment eliminated residual toluene. The product was dissolved in acetone (100 ml) and aliquots were taken for determination of yield by weight and titration. The balance of the material was freed of solvent and esterified with distilled diazomethane (18) in ether solution. The ether was removed and the product examined by gas–liquid chromatography. The results are presented in Table I.

The steam distillate was extracted with hexane (100 ml). The hexane solution was dried over sodium sulphate, and after removal of solvent the monocarboxylic acids were converted to methyl esters with diazomethane. Analysis of these methyl esters is presented in Table II.

The methanol in the cold trap was diluted with water and extracted with hexane. The bulk of the solvent was removed and the remaining solution examined by gas–liquid chromatography. Only traces of nonaldehyde were observed.

2. Ozonolysis in Acetic Acid and Other Solvents

Ozonolyses were carried out as described above employing 40 ml of a mixture of acetic acid and methyl formate (3:1), acetone, or methyl formate, with cooling in an ice-water bath. After complete ozone absorption had ceased, aqueous hydrogen peroxide (30%, 5 ml) was added and the flask was fitted with a reflux condenser. After standing overnight at room temperature, the mixtures were heated on a steam bath for 1 hour.

TABLE I

Dicarboxylic acids obtained from ozonolysis of oleic acid and control oxidations

Experiment			% yield		Weight% dicarboxylic acid composition*							% other material‡
Run	Material	Solvent	Weight†	Titration†	C_9	C_8	C_7	C_6	C_5	C_4	C_3	
1-A	Oleic	CH_3CO_2H	96.2	77.5	70.6	7.1	6.1	3.8	4.7	6.9	0.8	24.1‖
1-B	Oleic	$(CH_3)_2CO$	83	73	86.8	8.2	1.2	1.1	1.1	1.6	—	20.7§
2-A	Oleic	MeOH	100	93.5	97.2	1.9	0.4	0.2	0.2	0.1	—	5.6‖
2-B	Oleic	MeOH	100	93.1	97.5	2.2	0.1	0.1	0.1	—	—	3.1‖
2-C	Oleic	MeOH	100	95.5	98.7	0.9	0.1	0.1	0.1	0.1	—	1.8‖
2-D	Oleic	MeOH	106	100	97.6	1.9	0.1	0.1	0.1	0.1	—	3.9‖
2-A	Oleic	MeOH			96.0	2.5	0.6	0.3	0.3	0.3	—	5.4§
2-B	Oleic	MeOH			95.4	2.9	0.7	0.4	0.2	0.4	—	4.2§
2-C	Oleic	MeOH			97.9	1.5	0.1	0.1	0.2	0.2	—	0.7§
2-D	Oleic	MeOH			97.8	1.5	0.2	0.2	0.2	0.1	—	1.5§
3-A	Azelaic	HCO_2H	97.7	98.2	98.9	1.0	—	—	0.1	—	—	1.1‖
3-A	Azelaic	HCO_2H			98.5	1.2	0.1	—	0.1	0.1	—	1.1§
3-B	Azelaic	HCO_2H	98.6	100.3	99.1	0.6	0.1	—	0.1	0.1	—	0.6§

*% of total dicarboxylic acids indicated by gas–liquid chromatography.
†Calculated as azelaic acid.
‡% of total volatile materials indicated by gas–liquid chromatography.
§Polyester substrate.
‖Silicone grease substrate.

TABLE II

Monocarboxylic acids from ozonolysis of oleic acid

Run†	% monocarboxylic acids	Weight% monocarboxylic acid composition*			% other material‡
		C_9	C_8	C_7	
2-D	95.8§	98.1	1.6	0.3	4.2
	95.7‖	98.4	1.2	0.4	4.3

*% of total monocarboxylic acids indicated by gas–liquid chromatography.
†Details set forth in Table I.
‡% of total volatile materials indicated by gas–liquid chromatography.
§Silicone grease substrate.
‖Polyester substrate.

Monocarboxylic acids were removed by extraction with hexane (100 ml) after addition of water (100 ml). The aqueous solution containing the dicarboxylic acids was freed of solvents and volatile acids as described above, yields were determined, and the product was converted to methyl esters. Gas–liquid chromatographic analyses of the products are presented in Table I for the acetic acid and acetone products. The methyl formate product did not differ appreciably from the acetone product.

3. Control Oxidation of Azelaic Acid

Pure azelaic acid (100% by gas–liquid chromatography of the dimethyl ester) was refluxed for 1½ hours with hydrogen peroxide solution in formic acid in the proportions used in the ozonolysis procedure. After removal of the solvent the recovery was 97.7% by weight. The esters were examined by gas–liquid chromatography and gave dicarboxylic acid chromatograms almost identical with those obtained from ozonolysis (Table I). The only significant feature appears to be the lower amounts of pimelic and adipic acids formed and the absence of the alcohol by-product.

4. Control Oxidation of Azelaic Acid Methyl Ester Semialdehyde Acetal

Azelaic acid methyl ester semialdehyde was prepared from methyl oleate by ozonolysis in acetic acid and reduction with zinc (8, 9). After a preliminary distillation, a concentrate

was converted to the acetal by refluxing with methanolic HCl (46). The acetal methyl ester was treated with sodium bisulphite solution and after removal of solvent was purified by preparative gas–liquid chromatography (47). The product was not completely pure (cf. ref. 8), but on treatment with hydrogen peroxide in formic acid, a vigorous exothermic reaction took place, and working-up of the products, as in section 2 above, gave a product the gas–liquid chromatogram of which was virtually identical with that obtained by oxidation of azelaic acid.

5. Determination of Peroxidic Materials

An aqueous solution of hydrogen peroxide (30%, 5 ml) was added to the solvent under consideration (40 ml) in a round-bottomed standard taper flask fitted with a reflux condenser. The solution was heated on a steam bath, aliquots being withdrawn at intervals for analysis. Determination of hydrogen peroxide was made with ceric sulphate, and of peracids, with potassium iodide and sodium thiosulphate (48). Only formic acid showed an appreciable peracid content at any time, and only with formic acid or mixtures containing formic acid was rapid destruction of all active oxygen evident.

6. Gas–Liquid Chromatography

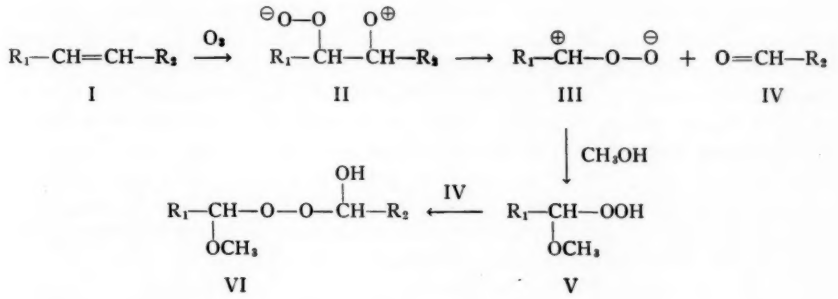
The apparatus employed was a Podbielniak Chromacon. Columns were 10 ft in length and $\frac{1}{4}$ in. in diameter, packed with 30% of either high-vacuum silicone grease (Dow Corning), especially treated (49), or diethylene glycol – adipic acid polyester (LAC-2-R-446), on 60–80 mesh Chromosorb. Analyses of monocarboxylic esters were carried out at 100° C with hydrogen flow rates of 55 ml/min for the polyester column and 100 ml/min for the silicone column. Dicarboxylic esters were analyzed at 160° with hydrogen flow rates of 85 ml/min for the polyester column and 38 ml/min for the silicone column. Areas were determined by a ball-and-disk-type integrator. The errors associated with the small peaks ($\sim$0.1%) may be $\pm$30% owing to the few counts available (50).

The monocarboxylic acid chromatograms showed, in addition to minor amounts of octanoic and heptanoic methyl esters, three other components. The minor one was identified as nonanal and the others as octanol and octyl formate, the latter predominating. These identifications are assigned on the basis of complete coincidence of small additions of authentic materials added to the ozonolysis product, carried out on both polyester and silicone substrates.

In addition to a large number of very minor peaks, the dicarboxylic acid ester chromatograms showed one major non-dicarboxylic acid by-product. Methyl 8-hydroxyoctanoate was not available, but methyl 9-hydroxynonanoate was prepared by the method of Diaper and Mitchell (51). Calculations of the equivalent chain lengths (52) of methyl 8-hydroxyoctanoate were made from the retention times of methyl 9-hydroxynonanoate. These indicated that the retention times, relative to dimethyl azelate, would be 0.605 on the silicone column and 1.16 on the polyester column. Minor peaks were in fact observed at 0.610 and 1.20 respectively. The formyl ester of the alcohol methyl ester could be similarly shown to have relative retention times, calculated from those of the formyl ester of methyl 9-hydroxynonanoate, of 0.680 on the silicone grease column and 0.905 on the polyester column. These values correlate with the relative retention times of the major non-dicarboxylic acid product, 0.706 and 0.902 respectively. The value of the relative retention time for the by-product peak on the silicone grease column could not be accurately determined as it was overlapped by the dimethyl suberate peak. Mixed runs with small amounts of methyl azelaaldehyde (9) did not coincide with any component on either silicone or polyester analyses.

DISCUSSION

The Criegee ozonolysis mechanism (1, 53, 54) suggests that the zwitterion (III) in the presence of a reacting solvent, in this case methanol, leads to a methoxy hydroperoxide (V). There is a possibility that this may then react with the aldehyde (IV) to give a hemiperacetal (VI). If the latter step were not complete (cf. ref. 33), some loss of volatile aldehyde would be expected when the methanol is removed under vacuum, whereas only a trace of free aldehyde (nonaldehyde) has been observed in this step. Reaction of the aldehydes with methanol to give hemiacetals or acetals is also a possibility (37).



The decomposition of the hydroperoxides, hemiperacetals, hemiacetals etc. is readily accomplished by treatment with hydrogen peroxide and formic acid, the active agent probably being performic acid (55) formed *in situ*. Peracetic acid has been employed in similar cases (15, 16, 37). Performic acid decomposes readily on heating, ensuring the rapid destruction (31, 32) of all peroxidic materials, to leave inert products. Regardless of the nature of the material being oxidized, less than 1% nonaldehyde and only traces of azelaic semialdehyde are found in all cases. This can be taken as a reliable indication that oxidation is virtually complete.

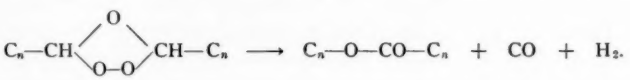
The quality of the reagents is significant. Thus results 2-A and 2-B, Table I, where ordinary reagent grade methanol was employed, show slightly higher amounts of both dicarboxylic acid and non-dicarboxylic acid by-products than results 2-C and 2-D where spectrophotometric grade methanol was used.

The acidic composition of the monocarboxylic acid fraction (Table II) parallels, in certain respects, that of the dicarboxylic acid product. However, since the recovery is only about 90% and losses of lower materials are high, the results are less significant.

The close duplication of the ozonolysis results by the control oxidations of azelaic acid and azelaic acid methyl ester semialdehyde acetal (see Table I, also above), not only in terms of secondary dicarboxylic by-products, but also partly in the miscellaneous by-products, indicates that these materials result from true secondary oxidation by the hydrogen peroxide in formic acid, and do not form during the ozonolysis step.

The occurrence of the presumed alcohol by-products in both the monocarboxylic and dicarboxylic acid products suggests a symmetrical reaction characteristic of isolated double bonds, and since they are not found in the control oxidation reactions (see above), their formation must take place during the ozonolysis or decomposition of the ozonide. Two possible mechanisms may be considered. In the first instance Pasero *et al.* (56–58)

have observed the formation of appreciable amounts of esters during the ozonolysis of monoethylenic acids, and ascribe this to simple decomposition of the ozonide:



Thus monooctyl azelate is one of the two potential products in the ozonolysis of oleic acid, and in the presence of formic acid and water, might be expected to hydrolyze or transesterify to give both octanol and octyl formate, together with azelaic acid.

Lefort *et al.* (59, 60) have observed the formation of similar esters (C_n—O—CO—C_n) when aliphatic peracids are heated, although under anhydrous conditions, suggesting a complex reaction of peracids during the ozonide decomposition with hydrogen peroxide in formic acid. On the present evidence either mechanism might be applicable.

Complete elimination of by-products is a highly desirable but rarely attained end in all oxidative fission procedures. The methanol – formic acid procedure reduces the by-product level to a point where gas–liquid chromatography is greatly facilitated, while at the same time retaining the advantage of total recovery of the dicarboxylic acids. The by-products do not, therefore, detract from the advantages of this technique, combined with gas–liquid chromatography, as a quantitative means of analysis of the position of ethylenic double bonds in long-chain monounsaturated fatty acids.

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THE CRYSTAL STRUCTURE OF 1,3-DICHLORONAPHTHALENE[1]

J. TROTTER

ABSTRACT

Crystals of 1,3-dichloronaphthalene are monoclinic with four molecules in a unit cell of dimensions $a = 15.11$, $b = 3.92$, $c = 14.86$ Å; $\beta = 96.0°$; space group $P2_1/n$. The x and z parameters of the chlorine and carbon atoms have been determined from Patterson and Fourier projections along the b-axis. The bond distances in the molecule, determined from the lengths projected on (010) together with estimated orientation angles, are very similar to the corresponding distances in naphthalene.

INTRODUCTION

One of the molecules being studied during investigations of the crystal structures of some naphthalene derivatives was previously reported as 1,2-dichloronaphthalene (1). During the course of the analysis, however, it became evident that the b-axis Patterson projection corresponded not to 1,2-dichloronaphthalene, but to the 1,3-derivative, and melting-point studies revealed that, while the main bulk of the sample was rather impure 1,2-dichloronaphthalene, the well-formed sublimed crystals used in recording the X-ray data were pure 1,3-dichloronaphthalene (2). The present communication gives details and results of the analysis of these sublimed crystals.

EXPERIMENTAL

Crystals of 1,3-dichloronaphthalene, which were formed by sublimation over a period of several months, consist of colorless prisms elongated along the b-axis. The density was measured by flotation in aqueous potassium iodide solution. The unit-cell dimensions and space group were determined from rotation and oscillation photographs of a crystal rotating about the b-axis, $h0l$ and $h1l$ Weissenberg films, and $hk0$ and $0kl$ precession films.

Crystal Data

1,3-Dichloronaphthalene: $C_{10}H_6Cl_2$; molecular weight = 197.1; melting point = 61° C.

Monoclinic; $a = 15.11 \pm 0.04$, $b = 3.92 \pm 0.01$, $c = 14.86 \pm 0.04$ Å; $\beta = 96°00' \pm 10'$.

Volume of the unit cell = 875.4 Å^3.

Density: calculated (with $Z = 4$) = 1.495 g cm^{-3}, measured = 1.494 g cm^{-3}.

Absorption coefficient for X rays: $\lambda = 1.542$ Å, $\mu = 60.6$ cm^{-1}.

Total number of electrons per unit cell = $F(000) = 400$.

Absent spectra: $h0l$ when $(h+l)$ is odd, $0k0$ when k is odd. Space group is $P2_1/n$—C_{2h}^5.

The intensities of the $h0l$ reflections[2] were recorded on Weissenberg exposures for a crystal rotating about the b-axis, using Cu $K\alpha$ radiation, with multiple-film technique to correlate strong and weak reflections. The intensities were estimated visually, the range being about 1500 to 1. The crystal cross section normal to the b-axis was fairly uniform, and no absorption corrections were applied. The structure amplitudes were derived by

[1]*Manuscript received June 12, 1961.*
Contribution from the Department of Chemistry, University of British Columbia, Vancouver 8, B.C.

[2]*The $\bar{1}01$ reflection was cut off by the beam stop.*

the usual formulae for a mosaic crystal, the absolute scale being established later by correlation with the calculated structure factors. Two hundred and twenty-nine independent $h0l$ reflections were observed, representing about 78% of the possible number observable with Cu $K\alpha$ radiation.

STRUCTURE ANALYSIS

The b-axis Patterson projection exhibited no peak corresponding to the Cl–Cl vector in a 1,2-derivative. Taking the two largest peaks on the map as corresponding to Cl–Cl interactions, positions were derived for the two chlorine atoms in the asymmetric unit, and by setting the origin of the Patterson function in turn at these two positions and at the positions related to them by the center of symmetry at the origin of the cell, a vector convergence (minimum) function was plotted. This map indicated quite clearly that the molecule was 1,3-dichloronaphthalene, all 10 carbon atoms being well-resolved, and co-ordinates were obtained for all the chlorine and carbon atoms. Structure factors were calculated for all the observed $h0l$ reflections, using the scattering factor for chlorine of Tomiie and Stam (3) with $B = 4.8$ Å^2, and the curve of Berghuis *et al.* (4) for carbon with $B = 4.8$ Å^2. The discrepancy factor was 28.2%.

Refinement proceeded by computing successive $(F_o - F_c)$ syntheses, and by altering the positional and temperature parameters to minimize the slopes and difference electron densities at the atomic centers. After two cycles the R value had been reduced to 15.5%; observed and calculated structure factors are not listed, but are available from the author. The final F_o synthesis, computed with measured structure amplitudes and calculated signs, is shown in Fig. 1.

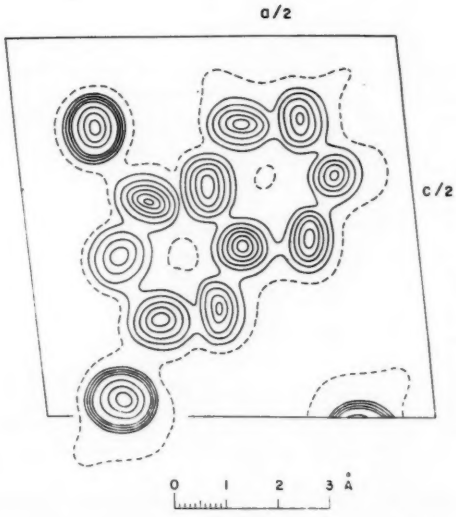


FIG. 1. Electron-density projection along the b-axis. Contours at intervals of 1 e Å^{-2} except at the chlorine atoms, where contours above 5 e Å^{-2} are at intervals of 5 e Å^{-2}; the one-electron line is broken.

No effort has been made to determine the y co-ordinates, since three-dimensional methods would be required, and it is not considered that the problem is sufficiently important to justify the great amount of work which would be involved.

Co-ordinates and Bond Distances

The final positional parameters are listed in Table I, referred to the crystal axes and

TABLE I

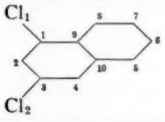
Final positional parameters

Atom	x	z
Cl_1	.1016	.1308
Cl_2	.0991	−.2315
C1	.159	.037
2	.119	−.045
3	.159	−.126
4	.241	−.111
5	.369	−.018
6	.409	.069
7	.371	.145
8	.289	.137
9	.246	.052
10	.281	−.025

expressed as fractions of the unit-cell edges. The projected bond distances calculated from these co-ordinates are given in the second column of Table II. The inclinations of

TABLE II

Bond distances (Å) and orientation angles (θ)



Bond	Projected bond distances, d_{proj}	θ	Bond distances, $d = \frac{d_{proj}}{\cos\theta}$	Mean bond lengths	Naphthalene bond lengths
1—2	1.299	15°	1.342	1.36	1.36
5—6	1.375		1.421		
3—4	1.234	23°	1.342		
7—8	1.238		1.346		
4—10	1.362	15°	1.407	1.42	1.43
8—9	1.357		1.402		
5—10	1.331	23°	1.447		
1—9	1.321		1.436		
2—3	(1.401)	22°	(1.513)	1.42	1.42
6—7	1.313		1.418		
9—10	1.308	22°	1.413	1.41	1.41
Cl_1—1	1.716	13°	1.76	1.76	—
Cl_2—3	1.722	12°	1.76		

the various sets of parallel bonds to the plane of projection, the true bond lengths, and the mean distances were derived as for 1,4-dibromonaphthalene (5), and the values are included in Table II. The agreement between the mean lengths and those in naphthalene (6, 7), which are listed in the final column of the table, is extremely good, indicating that the mean distances are more accurate than the individual values.

Comparison of the inclination angles for the C—Cl bonds and for parallel C—C bonds indicates that Cl_1 is displaced from the naphthalene plane by 0.28 Å. This displacement

is probably real, and is a result of steric interaction with the *peri* hydrogen atom. The deviation of Cl_2 from the aromatic plane is smaller (0.09 Å) and probably not significant.

Projected distances indicate that all the intermolecular contacts correspond to normal van der Waals interactions.

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RADICAL RECOMBINATION REACTIONS

PART II. TRIFLUOROMETHYL AND HEPTAFLUORO-*n*-PROPYL RADICALS[1,2]

G. O. PRITCHARD,[3] G. H. MILLER,[3] AND J. R. DACEY[4]

ABSTRACT

Mixtures of hexafluoroacetone and perfluoro-di-*n*-propyl ketone were photolyzed, and the ratio of the rates of formation of the three fluorocarbons, $R_{C_4F_{10}}/R^{1/2}_{C_2F_6}R^{1/2}_{C_6F_{14}}$, was found to equal 1.77 ± 0.10 over a 107° temperature range.

INTRODUCTION

In this paper we are concerned with the photolytic cogeneration of $CF_3\cdot$ and $C_3F_7\cdot$ radicals in a gas-phase system, and the temperature dependence, if any, of the three recombination reactions:

$$CF_3\cdot + CF_3\cdot \xrightarrow{k_{1\text{-}1}} C_2F_6, \qquad [1\text{-}1]$$

$$CF_3\cdot + C_3F_7\cdot \xrightarrow{k_{1\text{-}2}} C_4F_{10}, \qquad [1\text{-}2]$$

$$C_3F_7\cdot + C_3F_7\cdot \xrightarrow{k_{2\text{-}2}} C_6F_{14}, \qquad [2\text{-}2]$$

with respect to each other.

In a steady-state condition we have

$$k_{1\text{-}2}/k^{1/2}_{1\text{-}1}\,k^{1/2}_{2\text{-}2} = R_{C_4F_{10}}/R^{1/2}_{C_2F_6}R^{1/2}_{C_6F_{14}}, \qquad [I]$$

provided these reactions are the only sources of the three fluorocarbons.

At the pressures used there are no third-body restrictions affecting the recombinations, and the small temperature dependence associated with the collision number will cancel out in expression [I]. Thus any temperature variation of this ratio must be due to differing activation energies for one or all of the processes.

EXPERIMENTAL

The apparatus has essentially been described previously (1). The 3130-Å radiation was collimated by a single quartz lens and a stop, passed through a blue glass (Corning 9863), and into a 157-ml, cylindrical, 10-cm-long quartz reaction cell, fully illuminating it. The cell was surrounded by an outer steam jacket. Refluxing liquids used to attain temperatures other than 100° were xylene, 140°; *o*-dichlorobenzene, 180°; and tetralin, 207°.

The radical sources were the two ketones, $(CF_3)_2CO$ and $(C_3F_7)_2CO$, purified as described previously (2, 3). No detectable impurities were found in the mass spectra. Photolysis of the ketones individually in the temperature range of our experiments yields CO and the corresponding fluorocarbon in a 1:1 ratio via a free-radical mechanism (2, 3).

[1] *Manuscript received June 23, 1961.*
[2] *Contribution from the Chemistry Department, University of California, at Santa Barbara, Goleta, California, and the Chemistry Department, Royal Military College, Kingston, Ontario.*
[2] *For Part I of this series see reference 1.*
[3] *University of California.*
[4] *Royal Military College.*

A Toepler pump was attached to the reaction cell to ensure complete mixing of the ketones before irradiation. After photolysis, the CO was separated at −195° and measured in a gas burette. The remainder of the reaction mixture was condensed into a tube containing thoroughly outgassed distilled water. After the contents had warmed up, the tube was shaken vigorously and reattached to the vacuum system. Blank experiments showed that this procedure removed all of the $(CF_3)_2CO$ and most of the $(C_3F_7)_2CO$ as hydrates. C_2F_6 was then separated on Ward – Le Roy stills at −160°, and C_4F_{10} at −120°. The purity of all the products was checked mass spectrometrically. A fraction collected at −80° was slightly larger than that required by radical balance and contained C_6F_{14} and some unreacted $(C_3F_7)_2CO$. Perfluorohexane was identified unambiguously from the relative peak heights at masses 119 ($C_2F_5^+$), 131 ($C_3F_5^+$), and 169 ($C_3F_7^+$) in the mass spectrum (4).

The ultraviolet spectrum of the heavier ketone was taken on a Cary Model 14 recording spectrophotometer. There is strong absorbance between 2500 and 3600 Å, with structure between 3000 and 3500 Å. The absorbance maximum is at 3130 Å; molar extinction coefficient 32.0 l. mole^{-1} cm^{-1}. This is about four times as large as that for $(CF_3)_2CO$ at 3130 Å (2). Mixtures of the ketones gave additive spectra.

RESULTS

The data are collected in Table I. Due to the clear-cut nature of the photolysis mechanisms, C_6F_{14} has been calculated from CO − $(C_2F_6 + C_4F_{10})$. Consideration of runs 4 to 10 indicates the constancy of the ratio $k_{1\text{-}2}/k_{1\text{-}1}^{1/2}k_{2\text{-}2}^{1/2}$ over 107°, so that further experimentation did not appear worth while.

No experiments below 100° were envisaged due to the temperature and pressure dependence of the quantum yields, especially for $(CF_3)_2CO$, in this range. However, even at 100° in the early experiments (run 3 is given as an example), a suppression of $CF_3\cdot$ products was found. Ratios deduced from them were not considered reliable due to the small amounts of C_2F_6 obtained. Runs 1 and 2 give data for the ketones when they were photolyzed separately. The percentage decompositions are in accord with the high-pressure quantum yields, 0.6 for $(CF_3)_2CO$ and 1.0 for $(C_3F_7)_2CO$ at 100°, and the enhanced absorbance of the heavier over the lighter ketone at 3130 Å. Examination of runs 3, 4, and 5 shows suppression of the $(CF_3)_2CO$ photodecomposition with increasing $(C_3F_7)_2CO$ concentration. This can only be due to the very efficient deactivation of the excited species $(CF_3)_2CO^*$ by the heavier ketone. As expected, this effect lessens with rising temperature.[5] Decrease in the initial $(C_3F_7)_2CO$ concentrations enabled us to approximate to the ideal fluorocarbon product ratio of 1:2:1.

Percentage decompositions were calculated from the ratios $(C_2F_6 + \frac{1}{2}C_4F_{10})/(CF_3)_2CO$ and $[CO - (C_2F_6 + \frac{1}{2}C_4F_{10})]/(C_3F_7)_2CO$. On a time basis the latter are not entirely self-consistent as the voltage to the lamp was not stabilized. For the short period of a particular experiment constant light intensity can be assumed. In any case small sporadic intensity fluctuations are of no consequence. Expression [I] is not subject to stationary-state limitations, and will remain constant until either ketone is completely consumed. We conclude that expression [I] = 1.77±0.10.

[5] *The other possibility of losing* $CF_3\cdot$ *products,*

$$CF_3\cdot + (C_3F_7)_2CO \rightarrow [CF_3(C_3F_7)_2CO\cdot] \rightarrow C_3F_7\cdot + CF_3COC_3F_7,$$

would lead to excess $C_3F_7\cdot$ *products, which was not observed, and contrary to the experimental findings, the effect would increase with temperature as such reactions require an activation energy of about 6–7 kcal/mole (see reference 10).*

TABLE I

Run	Temp. (°C)	Time (sec)	Reactants (mole $cc^{-1} \times 10^6$)		Products (mole $\times 10^6$)				k_{1-2}	% decomposition	
			$(CF_3)_2CO$	$(C_3F_7)_2CO$	CO	C_2F_6	C_4F_{10}	C_6F_{14}	$\frac{k_{1-2}}{k_{1-1}^{1/2}\ k_{2-2}^{1/2}}$	$(CF_3)_2CO$	$(C_3F_7)_2CO$
1	100	600	2.24	—	20.1	20.0	—	—	—	5.72	—
2	100	300	—	1.38	46.7	—	—	—	—	—	21.6
3	100	150	4.30	1.89	32.9	0.64	4.90	27.4	1.17	0.47	10.0
4	100	150	4.81	0.687	19.7	6.10	9.16	4.44	1.76	1.41	8.36
5	100	150	4.38	0.860	26.3	1.95	9.26	15.1	1.71	0.96	14.6
6	100	600	2.06	0.430	58.6	5.85	24.1	28.7	1.86	5.54	60.3
7	140	300	3.65	0.543	38.1	6.57	17.5	14.0	1.82	2.67	26.7
8	180	150	2.15	1.29	25.9	1.24	8.05	16.6	1.78	1.56	10.2
9	180	300	4.81	0.637	50.1	13.3	23.2	13.6	1.73	3.30	25.2
10	207	300	3.61	0.668	46.6	11.7	21.6	13.2	1.74	3.97	23.0

DISCUSSION

(a) Pre-exponential Ratios

Assuming identical activation energies and steric factors for radical recombination reactions we have $k_{1\text{-}2}/k_{1\text{-}1}^{1/2}k_{2\text{-}2}^{1/2} = Z_{1\text{-}2}/Z_{1\text{-}1}^{1/2}Z_{2\text{-}2}^{1/2}$. By simple collision theory this reduces to approximately 2 in radical–radical systems. (The picture is somewhat more complicated if disproportionation reactions occur.) Using $\sigma CF_3\cdot = 4.0$ Å and $\sigma C_3F_7\cdot = 6.5$ Å, we obtain 2.2.

Kerr and Trotman-Dickenson (5) have shown that the experimental values for the combination of unlike radicals, in many cases, lie close to 2, and are independent of temperature, indicating that combination occurs on every collision. Calvert (6) has pointed out while this may be so for simple alkyl radicals, it is not true where one of the radicals is highly polar as a potential barrier to recombination exists. In the $CH_3\cdot + CF_3\cdot$ system (1) we noted a decrease from 3.0 to 1.8 over 140°, which was attributed to the polarity of the $CF_3\cdot$ radical, leading to a value of $E_{1\text{-}1} = 2$ kcal/mole. It has been pointed out (6, 7) that radicals formed in photolytic systems may not be thermally equilibrated, as evidenced in some cases by increasing disproportionation/recombination ratios with rising temperatures and falling pressure. If the disproportionation,

$$CF_3\cdot + CH_3\cdot \rightarrow CF_3H + CH_2\cdot,$$

occurred to a large extent, increasing with temperature, it could account for the observed decrease in $k_{1\text{-}2}/k_{1\text{-}1}^{1/2}k_{2\text{-}2}^{1/2}$. There was no positive evidence for it (1); also, the reaction has not been suggested in other $CH_3\cdot + CF_3\cdot$ systems (8, 9). The polar effect appears to be real.

Data from other similar systems are scant, as the determination of expression [I] was not the objective of the experiments. For $C_2H_5\cdot + C_3F_7\cdot$, it is 3.0, and admittedly scattered (5), and 2 ± 1 for $CH_3\cdot + C_2F_5\cdot$ (10), both ratios being temperature independent. Other gas-phase values at room temperature are 2.2 for $CF_2Cl\cdot + CF_2ClCF_2\cdot$ (11) and 1.9 for $CF_2Cl\cdot + CFCl_2\cdot$ (12).

Polar effects are being further studied by the cophotolysis of $(CH_3)_2CO$ and $(C_3F_7)_2CO$; the results will be presented in a later publication.

(b) Activation Energies

In the present system we have $E_{1\text{-}2} - \frac{1}{2}E_{1\text{-}1} - \frac{1}{2}E_{2\text{-}2} = 0$. The only reasonable assumption is that all three activation energies are equal. We may infer that there is a barrier to recombination of about 2 kcal/mole for all simple perfluoroalkyl radicals.

If we consider the system

$$X\cdot + R{-}H \rightarrow X{-}H + R\cdot$$

$$X\cdot + X\cdot \rightarrow X_2,$$

where $X\cdot$ is $CF_3\cdot$, $C_2F_5\cdot$, or $C_3F_7\cdot$, the activation energy of the first reaction will be determined mainly by the R—H and X—H bond strengths, and the repulsive forces between $X\cdot$ and R—H, and X—H and R. (13). As the activation energy for the second reaction remains constant, we may assume that the repulsive forces in the first do not vary significantly with $X\cdot$. If D(X—H) is constant, the activation energy should not vary with $X\cdot$.

Price and Kutschke (10) have summarized the available data for $CF_3\cdot$, $C_2F_5\cdot$, and $C_3F_7\cdot$ radicals. For CH_4 we have the following $E - \frac{1}{2}E$ values (±0.5 kcal): 10.3, 10.6, and 9.5 kcal, and for H_2: 8.8, 11.9, and 12.3 kcal. For $CF_3\cdot$ and $C_3F_7\cdot$ radicals only, we

have for D_2: 9.7 and 13.8 kcal, and for C_2H_6: 7.5 and 9.2 kcal. However, the last figure may be high by 1–2 kcal (14). Some correlation does exist, although $CF_3\cdot + H_2$ and $CF_3\cdot + D_2$ are exceptions.

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OCTAHALOGENODIBENZO-*p*-DIOXINS[1]

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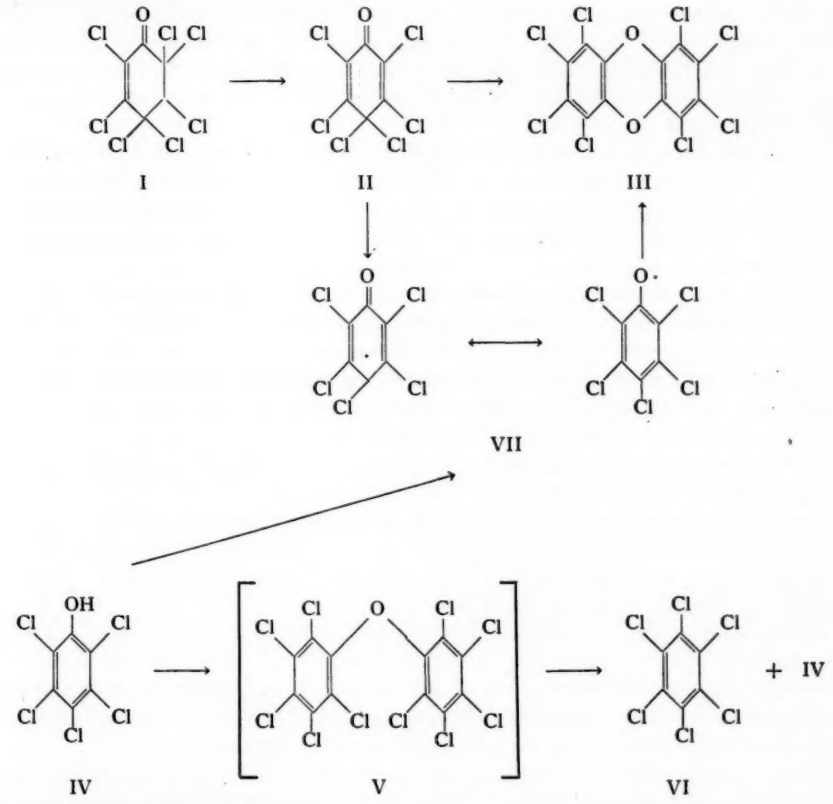
ABSTRACT

Pentahalogenophenols have been converted to 1,2,3,4,6,7,8,9-octahalogenodibenzo-*p*-dioxins in almost quantitative yields using the halogens or halogen-producing compounds as initiators of the reaction. It is suggested that an intermediate in this reaction is the pentahalogenophenoxy radical.

In 1872, Merz and Weith (1) heated the potassium salt of pentachlorophenol and obtained a high-melting compound which they called perchlorophenylenoxyd. This compound was also obtained when pentachloroanisole was treated with concentrated sulphuric acid (2). A few years later it was shown that when "α-heptachloroketotetrahydrobenzol" (I) was heated at 180°, it yielded 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) and when the temperature was raised over 200° the product was the "perchlorophenylenoxyd" (3, 4). Although the formula $(C_6Cl_4O)_x$ was assigned to "perchlorophenylenoxyd" there is little doubt that it was 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (III).



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About fifty years later, Denivelle and his co-workers (5) condensed a salt of pentachlorophenol with 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) in an inert solvent to form 4-*p*-chlorophenoxy-2,3,4,5,6-pentachloro-2,5-cyclohexadienone. This compound then underwent a number of reactions, including cyclization, to form 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (III).

Finally Sandermann, Stockmann, and Casten (6) were able to prepare III in poor yield directly from pentachlorophenol (IV) by heating it at 300°. The yield was poor because two simultaneous reactions occurred and the main product was hexachlorobenzene (VI). The formation of VI was explained in the following manner. Dehydration of two molecules of pentachlorophenol (IV) yielded the intermediate decachlorodiphenyl ether (V), which underwent cleavage in the presence of hydrogen chloride to form hexachlorobenzene (VI) and pentachlorophenol (IV). In the second reaction, two molecules of pentachlorophenol (IV) formed III with the elimination of two molecules of hydrogen chloride.

The writer has now developed a method for the conversion of pentachlorophenol (IV) to 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (III) in almost quantitative yield by heating the phenol in the presence of an initiator. First of all, the work of Biltz (4) was repeated and it was found that when 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) was heated over 200°, chlorine gas was liberated and the octachlorodibenzo-*p*-dioxin (III) was formed in high yield. From this observation the following mechanism of the reaction II → III became evident. Under the influence of heat, II dissociates to chlorine and the pentachlorophenoxy radical VII. Two of the radicals then associate, eliminate two chlorine atoms, and form III.

In view of the fact that hindered phenols can be oxidized to the corresponding phenoxy radicals (7, 8) it was decided to search for an oxidant which would convert pentachlorophenol (IV) to the pentachlorophenoxy radical VII and thus provide a method of preparing III from pentachlorophenol. 2,3,4,4,5,6-Hexachloro-2,5-cyclohexadienone (II) filled the requirements of such an oxidant because it not only liberated an oxidizing agent (chlorine) on being heated but also was converted to III. When an equimolecular mixture of pentachlorophenol (IV) and 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) was heated under reflux for 16 hours in 1,2,4-trichlorobenzene as solvent, or at 250–300° for 15 minutes without solvent, octachlorodibenzo-*p*-dioxin (III) was formed in 85% yield. Furthermore, the amount of II could be decreased to the point where almost catalytic quantities were sufficient to initiate the reaction of IV → VII → III. The reason for this becomes evident when one realizes that the chlorine liberated in the reaction VII → III can be used in the oxidation of IV to VII. An outside source of chlorine is necessary only to initiate the reaction and to replenish undue losses from the internal source. It is interesting to note that in the reaction VII → III, the ortho chlorines are eliminated, and little, if any, para elimination occurs. No poly(1,4-phenylene oxide) of the type described by Price (8) could be detected.

Although 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) is a very convenient initiator of the reaction IV → VII → III, it can be replaced by elementary halogens. When chlorine gas was bubbled slowly into a boiling solution of pentachlorophenol (IV) in 1,2,4-trichlorobenzene, the octachlorodibenzo-*p*-dioxin (III) was formed in 82% yield. Bromine and iodine were not as effective initiators as chlorine.

1,2,3,4,6,7,8,9-Octabromodibenzo-*p*-dioxin and 1,2,4,6,7,9-hexabromo-3,8-dichlorodibenzo-*p*-dioxin were prepared similarly from the corresponding phenols, showing that the method may have general application.

EXPERIMENTAL

2,3,4,4,5,6-Hexachloro-2,5-cyclohexadienone (II)

A reaction mixture of pentachlorophenol (500 g), *sym*-tetrachloroethane (400 ml), and iron powder (0.5 g) was stirred and heated at 90–100° while a slow stream of chlorine gas was passed in. When the weight increased by 80 g (12 hours) the dark reaction mixture was filtered and the filtrate concentrated *in vacuo* and allowed to cool overnight. The heavy, yellow crystals were filtered, washed with petroleum ether and with methanol, and dried. The yield from three successive crops was 444 g or 79%, and the product melted at 105–106°. The reported melting point is 106–107° (9).

1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (III)

(a) From 2,3,4,4,5,6-Hexachloro-2,5-cyclohexadienone (II)

The hexachlorocyclohexadienone (II) (20 g) was heated at 270–280° in an atmosphere of carbon dioxide for ½ hour. The resulting cake was crystallized twice from 1,2,4-trichlorobenzene, yielding white needles (11 g or 73%) melting at 330–332°. Anal. Calc. for $C_{12}Cl_8O_2$: C, 31.30; Cl, 61.75. Found: C, 31.74, 31.61; Cl, 62.26, 61.74. This compound sublimed readily *in vacuo* (12 mm pressure). It was very stable and crystallized from hot, concentrated sulphuric acid without charring.

(b) From Pentachlorophenol Using II as Initiator

A solution of pentachlorophenol (IV) (40 g), 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) (2 g), and 1,2,4-trichlorobenzene (80 ml) was heated under reflux for 16 hours. Hydrogen chloride and chlorine were evolved. The solution was allowed to cool and the white crystalline precipitate was filtered, washed with methanol, and dried. The product weighed 30 g (86%) and melted at 330°.

In another experiment an equimolecular mixture of pentachlorophenol (IV) and II was treated as above. The yield of III, based on the combined weights of II and IV, was 85%.

(c) From Pentachlorophenol Using Chlorine as Initiator

A solution of pentachlorophenol (IV) (25 g) in 1,2,4-trichlorobenzene (75 ml) was heated under reflux for 6 hours while chlorine gas was bubbled slowly into the reaction mixture. Then the refluxing was continued for another 10 hours without the introduction of chlorine. The resulting solution was allowed to cool, the white crystals were filtered, washed with methanol, and dried, m.p. 325–328°. The yield was 18 g or 83%.

(d) From Pentachlorophenol Using Bromine as Initiator

The experiment was carried out as in (*c*) but instead of the chlorine, 2 drops of bromine were added every 2 hours for the first 6 hours. The yield of III was 52%.

(e) From Pentachlorophenol Using Iodine as Initiator

The experiment was carried out as in (*c*) but instead of the chlorine, solid iodine (0.2 g) was added to the reaction mixture. Iodine vapors were visible in the reaction flask throughout the whole reaction time of 16 hours. The yield of III was 23%.

1,2,3,4,6,7,8,9-Hexabromodibenzo-p-dioxin

A mixture of pentabromophenol (35 g) and 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) (0.2 g) was heated at 310–320° for ½ hour and then at 360° for a few minutes. Hydrogen bromide and bromine were evolved. The cooled product, on crystallization from 1,2,4-trichlorobenzene, yielded white plates (15 g or 52%) melting at 403–406°. Anal. Calc. for $C_{12}Br_8O_2$: Br, 78.43. Found: Br, 78.95, 79.02.

2,3,5,6-Tetrabromo-4-chlorophenol

Bromine (320 g) was added dropwise over 6 hours to a stirred solution of *p*-chlorophenol (64 g) in *sym*-tetrachloroethane (200 ml) and iron powder (0.2 g), in a reaction flask fitted with a condenser, stirrer, and dropping funnel. The temperature was kept at 90–100° by heating the mixture. Then the dark reaction mixture was heated at 100–120° for 15 hours and allowed to cool. The white precipitate was filtered and crystallized from benzene. The product (135 g) melted at 210–212°. A small portion, when crystallized from benzene–methanol, melted at 213–214° (lit. (10) m.p. 215°). Anal. Calc. for C_6HClBr_4O: C, 16.20; H, 0.23. Found: C, 16.12, 16.79; H, 0.70, 0.80.

1,2,4,6,7,9-Hexabromo-3,8-dichlorodibenzo-p-dioxin

A mixture of 2,3,5,6-tetrabromo-4-chlorophenol (30 g) and 2,3,4,4,5,6-hexachloro-2,5-cyclohexadienone (II) (0.2 g) was heated at 300–310° for 15 minutes and then at 350° for 15 minutes. The cooled product was crystallized from 1,2,4-trichlorobenzene, yielding 15 g (62%) of almost white crystals melting at 385–388°. Anal. Calc. for $C_{12}Cl_2Br_6O_2$: C, 19.5. Found: C, 19.84, 19.75.

In another experiment, 1,2,4-trichlorobenzene was used as solvent. After the solution had been heated for 16 hours the reaction was not complete because a considerable amount of the starting 2,3,5,6-tetrabromo-4-chlorophenol was recovered, and the yield of the product melting at 385–388° was only 36%.

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α-KETOETHERS

II. INFRARED SPECTRA AND ROTATIONAL ISOMERISM OF PHENACYL ETHERS[1,2]

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ABSTRACT

Bifurcation of the carbonyl-stretching bands in the infrared spectra of several phenacyl ethers has been observed and is interpreted in terms of rotational isomerism.

The infrared spectra of α-haloketones have been the subject of intensive study during the past decade. As a result of the work of Jones, Corey, Brutcher, and others, the dependence of the carbonyl-stretching frequency of cyclic α-haloketones on their geometry has been elucidated (2). Recently Bellamy and his collaborators (3) have studied the infrared spectra of acyclic α-chloroketones and have observed that these frequently show the presence of two carbonyl-stretching bands. Jones and Spinner (4) have also observed such bifurcation in the spectra of certain other types of α-substituted ketones. We report here a cognate phenomenon in the case of several α-ketoethers.

The positions of the carbonyl-stretching bands of these compounds in carbon disulphide solution are shown in Table I, together with those of the parent ketones and of two

TABLE I

Carbonyl-stretching bands of ketoethers and their parent ketones

Compound						
No.	Formula	R	Source	$\lambda_{max}^{CS_2}$ (μ)[a]	Δλ (μ)	ϵ_1/ϵ_2[b]
1	$C_6H_5COCH_2R$	H	[c]	5.92		
2	"	OCH_3	[d]	5.86, 5.93	0.07	1.3
3	"	OC_2H_5	[d]	5.86, 5.93	0.07	1.2
4	"	OC_4H_9-*n*	[d]	5.86, 5.93	0.07	1.2
5	"	$OC_{10}H_{21}$-*n*	[e]	5.86, 5.93	0.07	1.2
6	"	OC_6H_5	[f]	5.85, 5.93	0.08	1.4
7	"	CH_2OCH_3	[g]	5.93		
8	"	$CH_2OC_2H_5$	[g]	5.92		
9	"	C_6H_5	[h]	5.91,[i] 5.94	0.03	0.77
10	$C_6H_5COCHC_6H_5$ $\vert$ OCH_3		[j]	5.89, 5.94	0.05	0.62
11	$C_6H_5CH_2COCH_3$		[c]	5.78,[i] 5.82	0.04	0.73
12	$C_6H_5CH_2COCH_2OCH_3$		[k]	5.78,[i] 5.80	0.02	1.1

[a]Concentration: ca. 2%.
[b]Ratio of apparent extinction coefficients of lower and higher wave-length bands.
[c]Commercial.
[d]See Experimental section.
[e]Prepared by Dr. T. J. Clark by the method of Newman and Beal (6).
[f]Ref. 1.
[g]Prepared by the method of F. Straus and A. Berkow. Ann. **401**, 121 (1913).
[h]Prepared by Dr. D. R. Moore by the method of D. A. Ballard and W. M. Dehn. J. Am. Chem. Soc. **54**, 3970 (1952).
[i]This band often appeared as a shoulder.
[j]Prepared by Dr. B. L. Shapiro by the method of E. Fischer. Ber. **26**, 2412 (1893).
[k]Cf. R. B. Moffett and R. L. Shriner. Org. syntheses, Coll. Vol. III, 562 (1955).

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Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts.
[2]*For Part I of this series see reference 1.*
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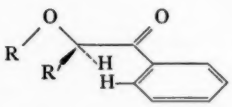
β-ketoethers. Several features of these data may be noted.

(i) The α-ketoethers of type $C_6H_5COCH_2OR$ (compounds 2–6) show a band close in position to the single band of acetophenone, together with a second band at lower wave length; the band separation of 0.07–0.08 μ is identical to that observed by Bellamy for compounds of type $ArCOCH_2Cl$ in solution in carbon tetrachloride.

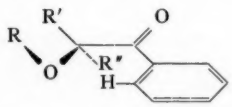
(ii) The two β-ketoethers of type $C_6H_5COCH_2CH_2OR$ (compounds 7 and 8) each show a single band close in position to that of acetophenone.

(iii) The remaining α-ketoethers (compounds 10 and 12) show splitting of the carbonyl band; the splitting is smaller, however, than that observed for the compounds of type $C_6H_5COCH_2OR$, and the parent ketones (compounds 9 and 11) also show a small splitting of their carbonyl bands.

The splitting of the carbonyl bands of the ketoethers of type $C_6H_5COCH_2OR$ can best be interpreted in terms similar to those suggested by Bellamy in the case of the phenacyl halides. The band at lower wave length is assigned to a rotational isomer in which the dipole associated with the CH_2—O—R group is in the same plane as that of the carbonyl group and acts in approximately the same direction, while the other band is assigned to a rotational isomer in which the CH_2—O—R dipole lies appreciably out of this plane. Although detailed interpretation in the present case is made more difficult by the greater multiplicity of rotational isomers, examination of scale molecular models indicates that the two types of rotational isomers most likely to be present are I and II. The isomer



I, R′ = H, R = alkyl or C_6H_5
VIII, R′ = C_6H_5, R = CH_3

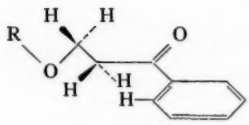


II, R′ = R″ = H, R = alkyl or C_6H_5
VI, R′ = C_6H_5, R″ = H, R = CH_3
VII, R′ = H, R″ = C_6H_5, R = CH_3

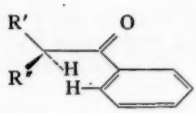
I in which the CH_2—O bond is cis to the C=O bond is considered to give rise to the carbonyl band at lower wave length because of dipole interaction, while the gauche isomer II is assigned the higher wave-length band, which is close in position to the band of acetophenone.* It was found that the spectra of these ketoethers in solvents of increased polarity show an increase in the relative intensity of the lower wave-length band, as would be predicted on the basis of these assignments, and as observed previously in the case of the phenacyl halides (3).

The occurrence of a single carbonyl-stretching band in the spectra of the two β-ketoethers, $C_6H_5COCH_2CH_2OR$, may be attributed both to the attenuation of the field effect of the CH_2—O—R dipole upon the C=O dipole with increasing separation and to the probable large preponderance of the single type of rotational isomer III. It is of interest that the CH_2—O—R dipole in this species is directed approximately in the trans sense relative to the C=O dipole and thus any residual field effect would tend to increase the wave length of its carbonyl-stretching band relative to that of acetophenone. The fact that the single band of these two β-ketoethers falls very close to that of acetophenone implies that either this residual effect is negligible or that it is compensated by an inductive effect, which would operate in the opposite sense (cf. refs. 3 and 4).

**House and Blaker (5) have interpreted the presence of two carbonyl bands in the infrared spectra of glycidic esters in related terms.*



III



IV, R′ = C_6H_5, R″ = H
V, R′ = H, R″ = C_6H_5

The splitting of the carbonyl band of the parent ketone, deoxybenzoin (compound 9), is again most probably due to the presence of two types of rotational isomers,* i.e. IV and V. The fact that the related α-ketoether (compound 10) shows two bands in closely similar positions to those of the parent suggests that it exists in carbon disulphide solution mainly as rotational isomers of types VI and VII, but not VIII, which is analogous to I and would be expected to give rise to a band at a significantly lower wave length.† Similar considerations apply to the cases of phenylacetone (compound 11) and the related α-ketoether, compound 12; here, the increased number of possible types of rotational isomers prohibits detailed discussion, but again the close similarity of the positions of the bands of the ketoether to those of the parent ketone suggests that rotational isomers in which the C—O—CH_3 group is disposed with respect to the C=O group, as in I and VIII, do not make a major contribution to the rotational isomer mixture.

EXPERIMENTAL

Sources and Purification of Compounds

The sources of the compounds, other than the α-alkoxyacetophenones, are given in Table I. The α-alkoxyacetophenones were prepared by the acid-catalyzed decomposition of α-diazoacetophenone in alcoholic solutions by a method exemplified by the case of α-butoxyacetophenone below. The compounds were purified by distillation, molecular distillation, or crystallization, and their physical properties were checked with those reported in the literature or with those of samples prepared by standard methods in our laboratory.

α-Butoxyacetophenone‡

α-Diazoacetophenone (0.500 g) was added to a solution of *p*-toluenesulphonic acid monohydrate (0.045 g) in butanol (10 ml) maintained under a nitrogen atmosphere. Gas evolution began immediately. The mixture was heated under reflux at 98° for 2.75 hours. The excess of 1-butanol was removed under reduced pressure and the residue was subjected to molecular distillation at 78° (2 mm). The product was obtained as a pale yellow liquid in 70% yield. A second molecular distillation provided a colorless sample whose infrared spectrum was indistinguishable from that of the once-distilled product. This was characterized as its 2,4-dinitrophenylhydrazone which was recrystallized from methanol and had m.p. 136–137°.

Anal. Calc. for $C_{18}H_{20}N_4O_5$: C, 58.06; H, 5.41; N, 15.05. Found: C, 58.33; H, 5.49; N, 15.02.

**Jones and Spinner (4) have previously reported the splitting of the carbonyl-stretching band of deoxybenzoin in carbon tetrachloride solution and attributed it to rotational isomerism.*

†Examination of scale molecular models suggests that steric factors might well favor the preponderance of isomers of type VI and VII over those of type VIII.

‡This method is a modification of that of Newman and Beal (6).

Infrared Spectra

The spectra were recorded with a Perkin–Elmer Model 21 spectrophotometer with NaCl prism and were calibrated against the atmospheric band at 5.88 μ.

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THE EFFECT OF THE AMBIENT OXYGEN ON THE ELECTRICAL PROPERTIES OF AN EVAPORATED FILM OF PENTACENE[1]

HARUO KURODA[2] AND E. A. FLOOD

ABSTRACT

The "oxygen effect" on the dark current as well as that on the photocurrent was investigated using thin evaporated films of pentacene, 1,2-benzpentacene, 1,2,8,9-dibenzpentacene, 6,13-diphenylpentacene, and 5,7,12,14-tetraphenylpentacene. No oxygen effect could be detected in the dark current.

From the photocurrent dependence on oxygen pressure and from the rate of photocurrent decay, it is concluded that additional charge carriers are produced on illumination by photo-excitation of a surface complex.

INTRODUCTION

Ambient gases exert a considerable influence on the surface photoconductivities and semiconductivities of a number of organic crystals. In the case of anthracene crystals, the electron "acceptor" type gases O_2, NO, SO_2, HCl, BF_3, etc. increase the photoconductivity while the donor gases NH_3, $(CH_3)_2O$, $(CH_3)N$, etc., which are usually less strongly adsorbed, decrease the photoconductivity (1).

Chynoweth has suggested that ambient oxygen forms a surface peroxide with anthracene which decreases the rate of recombination of the charge carriers (2). Bree and Lyons (3) have adduced evidence that unstable surface peroxides are formed on exposure of anthracene to oxygen and they have suggested that an "anthracene peroxide biradical" is largely responsible for the increased conductivity.

In previous papers we have shown that the semiconductivities of films of mesonaphthodianthrene formed by sublimation are increased markedly by exposure to oxygen (4, 5). It was further shown that oxygen produces a new conduction mechanism with a lower activation energy, and we have attributed the effect to the formation of a complex between oxygen and the surface molecules. A similar oxygen effect might be expected in the case of crystals of other aromatic molecules which are reactive to oxygen. However, although pentacene is relatively highly reactive towards oxygen and readily forms an addition compound when solutions of pentacene are exposed to air, oxygen has but little effect on the dark-current semiconductivity of pentacene films.

In what follows we present the results of some exploratory experiments on the effect of ambient oxygen on the electrical properties of "evaporated films" of pentacene and of some pentacene derivatives. As will be seen, the effect of oxygen on these films is quite different from its effect on the mesonaphthodianthrene film.

EXPERIMENTAL

The experimental procedures and the equipment used were essentially the same as described previously (5), except for the addition of the photoconductivity cell which is shown in Fig. 1. The evaporated films were prepared by high-vacuum sublimation onto a teflon substrate carrying two carbon electrodes which had been painted on previously using a water suspension of graphite (Aquadag). The evaporated films of hydrocarbon

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were of the order of a few microns in thickness. A tungsten-filament lamp was used as a light source. Direct-current voltage was applied between the two electrodes and the current through the film was measured by means of a Hewlett and Packard d-c. microvoltammeter model 425, together with a suitable recorder.

Pentacene, 1,2-benzpentacene,[3] and 1,2,8,9-dibenzpentacene[3] were obtained from L. Light and Company. 6,13-Diphenylpentacene and 5,6,12,14-tetraphenylpentacene were kindly supplied by Dr. R. N. Jones of the National Research Council, Ottawa.

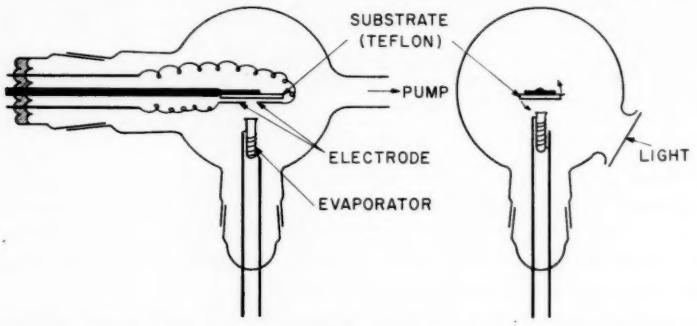

FIG. 1. Cell for the measurement of the oxygen effect on photoconduction.

RESULTS AND DISCUSSIONS

A. Dark Current

The temperature dependence of the dark current was determined, in nitrogen, with each film immediately after its preparation. In the temperature range 0° C to 150° C, the temperature dependence of the dark current was found to obey the exponential law

$$i_{\text{dark}} = i_0 \exp(-E/kT), \qquad [1]$$

where E is the activation energy for the semiconduction. No break was found in the $\log i$ vs. $(1/T)$ curves corresponding to any of the films of pentacene and pentacene derivatives studied. The values of the activation energies determined from experimental results are listed in Table I.

TABLE I

Activation energy of semiconduction

Compound	E (ev)
Pentacene	0.81
Diphenylpentacene	0.80
Tetraphenylpentacene	0.81
Dibenzpentacene	0.75
Benzpentacene	0.86

Although the reactivity of pentacene toward oxygen is as high as that of mesonaphthodianthrene, no appreciable change in either the dark current or the dark-current temperature dependence could be detected on exposure of pentacene films to oxygen. This

[3]*According to A. M. Patterson, L. T. Capell, and D. F. Walker ("The ring index". 2nd ed. American Chemical Society. 1960) the preferred names of these compounds are benzo[a]pentacene for 1,2-benzpentacene and dibenzo[a,l]pentacene for 1,2,8,9-dibenzpentacene.*

behavior is in marked contrast to the effect of oxygen adsorption on the dark current observed with mesonaphthodianthrene. Films of the other pentacene derivatives studied in this work behaved in a manner similar to that of pentacene films.

Of course we cannot conclude from this behavior that the surface reactions between these organic crystals and oxygen are purely physical adsorption or Van der Waals interactions, since the activation energy required to liberate a charge carrier from the surface complex could be higher than that of the semiconduction process in the bulk of the crystal or in the bulk of the microcrystalline mass. This seems not improbable in view of the reactivities of these pentacenes toward oxygen.

B. Change in Photocurrent on Exposure to Oxygen

The change in photocurrent of a freshly deposited pentacene film on exposure to oxygen is shown in Fig. 2. On exposure of the film to oxygen under 200 mm Hg pressure,

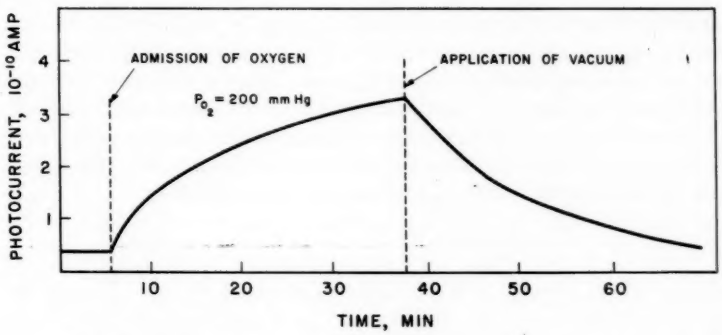

FIG. 2. Change in photocurrent through a pentacene film on exposure to oxygen.

the photocurrent rises slowly from the high-vacuum value and reaches a considerably higher stationary value in something over an hour. On evacuation, the photocurrent decreases, gradually approaching the original high-vacuum photocurrent.

Evidently the oxygen effect is reversible and the interaction between the surface and the oxygen molecule is not very strong. These rates of change are temperature dependent, the rates being higher at the higher temperatures.

Oxygen adsorption influences not only the magnitude of the photocurrent but also the rate of response to illumination. In the absence of oxygen the current through a pentacene film rises rapidly on exposure to light, taking less than a minute to reach its stationary value. In the presence of oxygen, however, the behavior is quite different. On exposure to light, the current rises rapidly to about the same value as in the case of the oxygen-free film, but the current continues to rise slowly until a considerably higher stationary value is obtained. When the light is extinguished, a rapid decrease in photocurrent is shown, the magnitude of its decrease being equal to that of the initial rapid rise; the photocurrent then decays slowly. The behavior is illustrated in Fig. 3. The rates of rise and fall of the current are temperature dependent, being higher at the higher temperatures.

If the photocurrent is observed with a light pulse of short duration (5–6 seconds) in place of the long continuous illumination, the height of the photocurrent pulse is not changed appreciably by exposure to oxygen. These facts suggest that two distinct

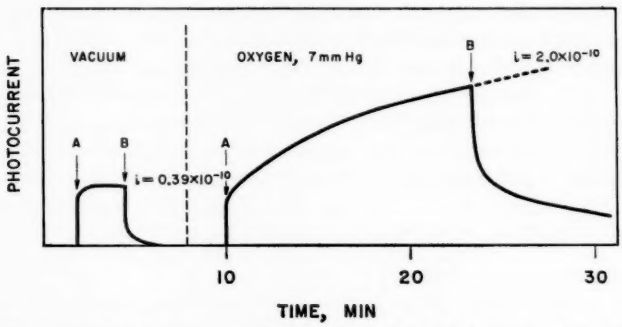

FIG. 3. Response of photocurrent to illumination. Light is admitted at A and shut off at B.

mechanisms are involved in the photoconduction process. In order to illustrate this situation more clearly, the following experiment was carried out. A pentacene film was exposed to oxygen for a long enough period for adsorption equilibrium to be attained. The film with ambient oxygen was then illuminated continuously until the stationary photocurrent was reached. At this point the continuous illumination was shut off and the sample subjected to short light pulses at 1-minute intervals. The photocurrent responses corresponding to the above experiment are shown in Fig. 4.

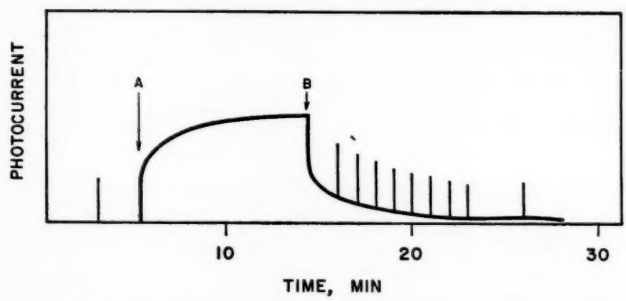

FIG. 4. Response of photocurrent to illumination in ambient oxygen. Continuous illumination is given between A and B.

It is interesting to note that the "heights" of the photocurrent pulses observed during the slow decay process are of almost the same magnitude as that of the pulse observed either for an oxygen-free film or for a film in oxygen before continuous illumination. This strongly suggests that the photocurrent observed in ambient oxygen consists of two more or less independent components, one of which has a short lifetime and is not sensitive to ambient oxygen while the other component has a relatively long lifetime and is oxygen sensitive. We shall represent the first of these photocurrent components by i_1 and the second, oxygen-sensitive component by $i_2(p)$, and write for the total photocurrent[4]

$$i = i_1 + i_2(p). \qquad [2]$$

[4]*Under our experimental conditions i_1 corresponds very approximately to an apparent conductivity of the order of 10^{-11} to 10^{-12}. The intrinsic conductivity of pentacene (30°) is of the order of 10^{-14}; anthracene, 10^{-15}; carbons, 10^{-7} to 1; graphite, $>10^3$.*

Films of 1,2-benzpentacene, 1,2,8,9-dibenzpentacene, and 6,13-diphenylpentacene exhibit oxygen-sensitive photoconductivities which are quite similar to those of the pentacene films.

Molecules in the pentacene crystal are closely packed as they are in crystals of anthracene and tetracene; hence, it is unlikely that the oxygen molecule can penetrate deeply into these crystals. In the case of aromatic molecules having substituent groups as large as rubrene (6,5,11,12-tetraphenylnaphthacene) we might expect a somewhat different situation, and, in fact, it is known that photooxidation can take place in the bulk of the rubrene crystal (6). Although the phenyl groups in 5,7,12,14-tetraphenylpentacene are not adjacent, as they are in rubrene, we might expect the crystals of the tetraphenylpentacene to be also rather loosely packed and that a transannular peroxide could be formed either by direct oxidation or by photooxidation. On this basis one might expect the "oxygen effect" on the photoconductivities of tetraphenylpentacene films to be larger than those on films of the other pentacenes. However, surprisingly enough, no oxygen effect could be detected at all, either in the photocurrent or in the dark current when films of 5,7,12,14-tetraphenylpentacene were examined experimentally.

While in solution, pentacene can be oxidized by exposure to oxygen, forming a transannular peroxide; in the case of the solid, the oxygen molecule cannot penetrate the closely packed crystal and appreciable oxidation of the solid does not normally occur on exposure to oxygen. It seems, therefore, safe to assume that under our experimental conditions, "oxide" formation is limited to the surface of the pentacene crystal. Thus in equation [2], i_1 is considered to be bulk photoconductivity while $i_2(p)$ is considered to be associated with surface states and hence sensitive to surface "oxide" formation.

C. Stationary Photocurrent and Oxygen Pressure

The stationary photocurrent determined after a long, continuous illumination is dependent on the oxygen pressure, as shown in Fig. 5.

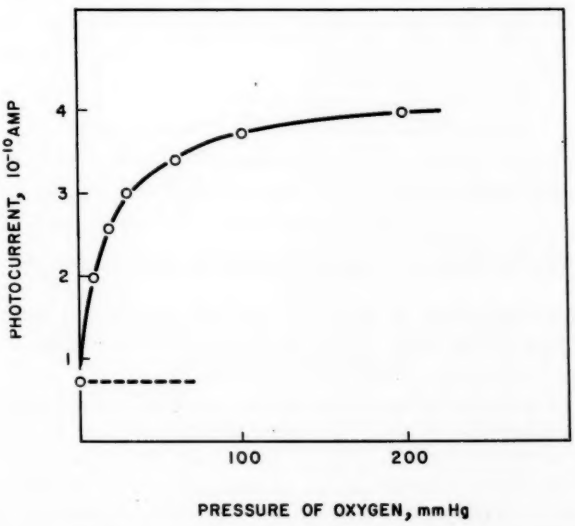

FIG. 5. Dependence of photocurrent on oxygen pressure.

Assuming that the adsorption of oxygen can be expressed by means of a Langmuir-type equation, we can write,

$$\frac{\theta}{1-\theta} = k_1 p, \tag{3}$$

where θ is the fractional surface area covered with oxygen at the pressure p, and k_1 the appropriate proportionality constant. When it is assumed that

$$i_2(p) = k_2\theta, \tag{4}$$

then

$$\frac{k_2}{i_2(p)} = (1/k_1)(1/p)+1 \tag{5}$$

and

$$i_2(p) = \Delta i(p) = i_{\text{photo}}(p) - i_{\text{photo}}(0). \tag{6}$$

Thus equation [5] can be expressed as

$$(1/\Delta i) = (1/k_1k_2)(1/p) + (1/k_2), \tag{7}$$

i.e., $1/\Delta i(p)$ is a linear function of the reciprocal of the oxygen pressure. In Fig. 6 the

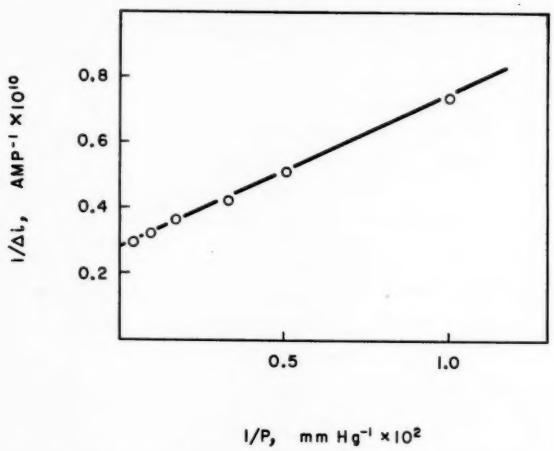

FIG. 6. Plot of reciprocal of photocurrent against reciprocal pressure.

reciprocals of the observed values of $\Delta i(p)$ are plotted against the reciprocals of the oxygen pressures and, as will be seen, $1/\Delta i(p)$ is indeed a linear function of $1/p$. Thus the evidence seems to indicate the validity of equation [4].

A relation similar to that of equation [7] has been reported for the effect of ambient gases on the photoconductivity of the anthracene crystal and has been attributed to the change in surface mobility of the charge carriers caused by adsorption (1). Recently Rosenberg (7) has also attributed the effect of oxygen on the photoconductivity of β-carotene to increased mobilities of charge carriers. While an increase in photocurrent caused by adsorption can be attributed either to an effective increase in the number of

charge carriers, or to an increase in their mobilities, or to a combination of both these mechanisms, the proportionality between $i_2(p)$ and θ can be explained much more directly if we assume the photoinjection of additional charge carriers from the surface complex. Further, this latter mechanism leads quite naturally to an explanation of the influence of ambient oxygen on the rates of the photocurrent responses.

D. Mechanism of Slow Photocurrent Decay

A photoconductive response of the type shown in Fig. 4 is usually associated with traps. As is well known, the decay of the photocurrent response in many cases can be described by an exponential decay law. The slow decay of Fig. 4, however, does not obey an exponential law.

If we assume that the photocurrent consists of two more or less independent components, namely bulk photocurrent and surface photocurrent, the decay will occur through the fast recombination in the bulk of the crystal, presumably according to an exponential decay law and this will be followed by the slower surface decay.

The oxygen molecule usually behaves as an electron acceptor toward the surface of a number of semiconductors and usually increases the electrical conductivity of p-type semiconductors, presumably by injection of additional p-type charge carriers. If the increase in the photocurrent of the pentacene films on exposure to oxygen is also due to the injection of additional p-carriers, as a result of photoinduced electron transfer to surface oxygen atoms, negatively charged surface complexes must remain on the surface and they will behave as traps for p-type carriers in the surface recombination process. This behavior would be consistent with the known chemistry of pentacene. On this basis, the decay of the photocurrent, after cessation of illumination, will take place by fast recombination in the bulk of the crystal and also by the relatively slow trapping of the injected p-type carriers by the negatively charged surface complexes, the two mechanisms being more or less independent.[5]

According to the model given above, the carriers at the tail of the photocurrent decay must be p-type carriers in the surface region and the number of these carriers must be equal to the net number of negatively charged traps on the surface. Thus for this part of the decay we can write

$$-\frac{dn}{dt} = Kn(N_t - n_t), \tag{8}$$

where N_t is the total number of negatively charged surface traps formed initially and n_t the number of trapped carriers. Hence $n = N_t - n_t$ and equation [8] becomes a second-order reaction equation, namely

$$-\frac{dn}{dt} = Kn^2 \tag{9}$$

or

$$1/n - 1/n_0 = Kt. \tag{10}$$

Since $i(t)$ is proportional to n, equation [10] can be written as

$$1/i(t) - 1/i(0) = K't,$$

[5] *If the bulk decay is truly exponential and very rapid, it suggests that the recombination is essentially a "trapping" mechanism in which the number of "traps" is relatively large and independent of the number of carriers. If this is indeed the case, these bulk recombination "traps" must be ineffective in trapping the additional p-type carriers.*

that is, $1/i(t)$ becomes a linear function of time. That this is actually the case may be seen from Fig. 7.

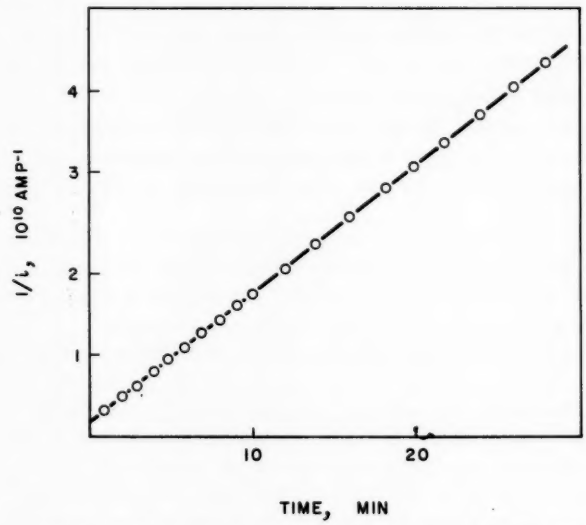

FIG. 7. Photocurrent decay in ambient oxygen (200 mm Hg).

While the above is a very unsophisticated description of the effect of oxygen on the photoconductivity of films of pentacene and some of its derivatives, both the dependence of the stationary photocurrent on the oxygen pressure as well as the decay of the photocurrent have been explained in terms of a very simple, plausible model, namely the formation of a surface "oxide" capable of providing additional *p*-type charge carriers on illumination. This model is reasonably consistent with the known tendency of some of these complex hydrocarbons to form biradical addition complexes and is reasonably consistent with the effect of oxygen on their absorption spectra. While, in the case of pentacene, the nature of the surface oxide is not known, the reversibility of the "oxygen effect" suggests that comparatively weak bonds are involved. Presumably the surface "oxide" is a "molecular" compound involving some resonance with a transient ionic form or biradical. On illumination, electrons are transferred and localized on the relatively immobile oxygen atoms, leaving unlocalized positive holes capable of acting as charge carriers.

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DEUTERIUM KINETIC ISOTOPE EFFECTS

V. TEMPERATURE DEPENDENCE OF β-DEUTERIUM EFFECTS IN WATER SOLVOLYSIS OF ISOPROPYL COMPOUNDS[1]

K. T. LEFFEK,[2] R. E. ROBERTSON, AND S. E. SUGAMORI

ABSTRACT

The secondary β-deuterium isotope effect (k_H/k_D) has been measured over a range of temperature for the water solvolysis reactions of isopropyl methanesulphonate, *p*-toluenesulphonate, and bromide. In these cases the isotope effect is due to a difference in entropies of activation of the isotopic analogues rather than a difference in the enthalpies of activation. It is suggested that the observed isotope effect is due to internal rotational effects of the methyl groups in the isopropyl radical, and the lack of an isotope effect on the enthalpy of activation is accounted for by a cancellation of an effect from this source and one from zero-point energy.

Secondary β-deuterium isotope effects in solvolytic reactions have been studied by several groups of workers (1–12). In all cases, replacement of hydrogen on β-carbon atoms of the alkyl radical by deuterium led to a reduction in the rate of solvolysis. The rate ratio (k_H/k_D) was found to vary from close to unity, for primary compounds (11, 12), to about 2.5, for tertiary compounds (1, 11). The source of this reduction in rate with deuterium substitution has usually been discussed in terms of the effect of changes in hyperconjugation associated with the activation process. The resulting weakening of C—H and C—D bonds implies a corresponding change in the difference in the zero-point energies. If this is the main source of the observed isotope effect, then an analysis of the temperature dependence of this effect should show a difference in the corresponding enthalpies of activation and any difference in the entropies and heat capacities of activation should be small. This explanation is in accord with the results reported by Shiner and Verbanic (2, 13) and by Lewis and Coppinger (5), who found in three solvolytic reactions that deuteration led to a change in both enthalpy and entropy of activation, the former being the major factor.

As a continuation of our general survey of β-deuterium isotope effects in the water solvolysis of alkyl halides and sulphonates (11), we have examined to what extent the above assumption is valid for the isopropyl system.

RESULTS AND DISCUSSION

Rate data over a range of temperature are given in Table I for the hydrolysis of isopropyl methanesulphonate, *p*-toluenesulphonate, and bromide, and their fully β-deuterated analogues. The rate data for the normal compounds were calculated from the three constant rate – temperature relationships previously determined in this laboratory (14). These relationships have been shown to reproduce the rate constants to better than the experimental error, and agreement between the rates so derived and experimentally determined for the protium compound was demonstrated for each of the above compounds, immediately prior to rate determinations on the deuterated

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TABLE I

Temperature dependence of the β-deuterium isotope effect for the solvolysis of isopropyl compounds in water

Compound	$10^4 k_H$ (sec^{-1})	$10^4 k_D$ (sec^{-1})	Temp. (°C)	k_H/k_D (±0.006)
Isopropyl methanesulphonate	3.691	2.386	30.001	1.54_7
	1.965	1.267	25.000	1.55_1
	1.019	0.6570	20.002	1.55_1
	0.3619	0.2340	12.514	1.54_7
	0.3612	0.2345	12.501	1.54_0
	0.1197	0.07698	5.000	1.55_5
Isopropyl *p*-toluenesulphonate	7.664	4.960	30.017	1.54_5
	7.650	4.942	30.001	1.54_8
	4.085	2.655	25.005	1.53_9
	4.082	2.648	24.999	1.54_2
	2.122	1.375	20.005	1.54_3
	1.071	0.6933	15.003	1.54_5
	0.5248	0.3415	10.003	1.53_7
	0.2900	0.1881	6.008	1.54_2
Isopropyl bromide	10.11	7.638	69.994	1.32_4
	10.10	7.670	69.993	1.31_7
	6.004	4.542	65.002	1.32_2
	3.486	2.645	60.000	1.31_8
	3.461	2.631	59.933	1.31_5
	1.981	1.509	55.005	1.31_3
	0.3123	0.2381	40.005	1.31_2
	0.3123	0.2371	40.005	1.31_7

compound. The rate ratios (k_H/k_D), given in the final column, are seen to be almost independent of temperature. The results were fitted to the transition-state relationship

$$[1] \qquad \log_{10}(k_H/k_D) = \frac{(\Delta H_D^{\ddagger} - \Delta H_H^{\ddagger})}{2.303RT} - \frac{(\Delta S_D^{\ddagger} - \Delta S_H^{\ddagger})}{2.303R}$$

by the method of least squares, and the resulting enthalpy and entropy differences are given in Table II. Thus the rate decrease resulting from β-deuteration in this system is

TABLE II

Changes in enthalpy and entropy of activation produced by β-deuteration in the solvolysis of isopropyl compounds in water

Compound	$(\Delta H_D^{\ddagger} - \Delta H_H^{\ddagger})$ ±std. error (cal mole^{-1})	$(\Delta S_D^{\ddagger} - \Delta S_H^{\ddagger})$ ±std. error (cal mole^{-1} deg^{-1})
Isopropyl methanesulphonate	7±28	−0.84±0.1
Isopropyl *p*-toluenesulphonate	−21±14	−0.93±0.05
Isopropyl bromide	−35±15	−0.65±0.05

due almost entirely to a difference between the entropies of activation of the isotopic analogues. This is in marked contrast to the earlier work and cannot be explained in terms of zero-point energy effects alone.

When equation [1] is used, it is assumed that the heat capacities of activation ($\Delta C_p^{\ddagger}$) for the protium and deuterium compounds are the same. Since it has been observed that $\Delta C_p^{\ddagger}$ is much more sensitive to changes in the anionic group than to changes in the alkyl radical (15), this assumption probably would not introduce a significant error in the general case. If such an error were detectable it would appear as a curvature in

a plot of $\log_{10}(k_H/k_D)$ versus T^{-1}. For the present case of the isopropyl compounds, $(\Delta H_D^{\ddagger} - \Delta H_H^{\ddagger})$ is approximately zero and, therefore, $(\Delta C_{p,D}^{\ddagger} - \Delta C_{p,H}^{\ddagger})$ must also be very close to zero (less than -0.5 cal mole^{-1} deg^{-1}).

Any explanation of the results in Table II must be capable of producing the entropy of activation difference and at the same time account for the lack of an enthalpy of activation difference. The probable sources of secondary deuterium isotope effects will be examined in the light of these requirements.

Zero-Point Energy Effects

It has been generally claimed that the formation of a positive charge in the transition state of a solvolytic reaction proceeding via a carbonium ion brings about an increase in hyperconjugation in the activation process. The vibrational frequencies of the β-C—H (or β-C—D) bonds are, therefore, reduced in the transition state relative to the initial state. As a result, the difference between the zero-point energies of the β-C—D bonds and the β-C—H bonds is less in the transition state than in the initial state, leading to a higher enthalpy of activation for the deuterated compound. This argument is well established (1–12) and was applied in an earlier paper (11) to isopropyl compounds solvolyzing in water. Our present results show that if such an effect is present in the isopropyl case, it must be cancelled by another enthalpy effect of the opposite sign. The earlier work of Shiner *et al.* (2, 13) and Lewis *et al.* (5) on the temperature dependence of the secondary deuterium isotope effect seems to indicate that, in general, enthalpy of activation differences do occur. It is concluded, therefore, that the lack of an enthalpy difference in our system is probably due to a fortuitous cancellation of two (or more) effects.

Solvation Effects

Specific solvation effects as a source of secondary β-deuterium isotope effects in solvolytic reactions were considered by Shiner (1, 2), although Lewis and Coppinger (5) and later Streitwieser *et al.* (7) produced evidence against such an explanation.

Indirect evidence against solvation effects as a major contributor to the isotope effect is also available from the derived kinetic parameters for hydrolysis reactions. Briefly, a consideration of the changes in the entropy of activation ($\delta_R \Delta S^{\ddagger}$) accompanying the structural change ethyl to isopropyl for a series of halides and sulphonates leads to the conclusion that no appreciable solvent reorganization has taken place about the alkyl group at the transition state (14). Even stronger evidence is the lack of significant differences in the solvent isotope effect (15). The lack of correlation between $\delta_R \Delta S^{\ddagger}$ and $\delta_R \Delta C_p^{\ddagger}$ is also consistent with this conclusion.

Finally, there is the evidence from the work of Maccoll *et al.* (17) that an isotopic effect very similar to those reported here has been observed in the absence of solvent. In the gas-phase pyrolysis of normal and β-deuterated isopropyl bromide, a reaction which is postulated to have a quasi-carbonium-ion transition state, an isotope effect $(k_H/k_D) = 2.5$ was obtained. This rate ratio was also not greatly dependent on temperature. The result, by its marked similarity to those results given in Table I, supports the conclusion that solvation effects are not an important source of the isotope effect in this system, although the evidence is not strong enough to rule them out entirely.

Internal Effects

Isotope effects from this source arise as a result of differences between the partition functions of the normal and deuterated molecules in the initial and transition states

(equation [2]). The partition function Q may be factorized into $Q_{trans} \times Q_{rot} \times Q_{vib} \times Q_{int\ rot}$.

$$[2] \qquad (k_H/k_D)_{int} = \frac{Q_H^{\ddagger}}{Q_H} \cdot \frac{Q_D}{Q_D^{\ddagger}}.$$

The maximum rate ratios which could arise from the translational and rotational partition function differences would occur if the initial state were unsolvated and the transition state highly solvated (11). For this extreme case the rate ratios are given by the equations

$$[3] \qquad (k_H/k_D)_{trans} = \left(\frac{M_D}{M_H}\right)^{3/2},$$

$$[4] \qquad (k_H/k_D)_{rot} = \left(\frac{\Pi I_D}{\Pi I_H}\right)^{1/2}.$$

In practice neither of these contributions to the isotope effect can be expected to exceed a few per cent. The contribution from the vibrational partition function is also small at the ordinary temperatures used in this study (18, 19).

The theory regarding internal rotation in molecules has remained less than adequate despite the considerable amount of research into the subject (20). Nevertheless, it has been recognized that changes in barriers to internal rotation during chemical reactions must be important in determining the thermodynamic reaction parameters (20). The isotope effects observed during this study can be accounted for semiquantitatively on the basis of the following two postulates concerning the barriers to internal rotation of the methyl groups in isopropyl compounds for the water solvolysis reaction. It is postulated: (*a*) that there is a considerable decrease in the barrier to rotation of the CH_3 (or CD_3) groups on going from the initial to the transition state and (*b*) that the barrier to rotation of the methyl groups is higher in the protium compound than in the deuterium compound in the initial state.

The justification of the first postulate is the empirical rule that sixfold barriers to rotation are considerably smaller than threefold barriers (20, 21). Examples can be found in methyl derivatives of benzene where the barriers to rotation of the methyl groups are about 0.5 kcal per mole (21). If it is accepted that the transition state in the water solvolysis of isopropyl sulphonates and halides approximates a planar carbonium ion, then the barrier will become sixfold during the activation process and hence will be greatly reduced in the transition state. The entropy of activation for the water solvolysis reaction becomes increasingly more positive along the series ethyl halide, isopropyl halide, tertiary butyl halide (16), a variation which is also in qualitative agreement with postulate (*a*).

Postulate (*b*) is based on a limited number of microwave spectroscopic measurements. Swalen and Costain (22) found that the barrier to internal rotation for the CH_3 group in acetone is approximately 6% higher than that for the CD_3 groups in acetone-d_6. In nitromethane, the barrier to internal rotation of the methyl group is 16% greater in the normal compound that in the deuterated compound (23). On the other hand, recent work on the barrier in deuterated ethanes, using infrared data, gives no clear indication that CH_3 groups have a higher barrier than CD_3 groups (24). Theoretical support for postulate (*b*) is not easily found since barriers to internal rotation are not well understood. Bartell (25) has recently drawn attention to the size effects among isotopic molecules as a possible explanation for secondary deuterium isotope effects. Since

hydrogen–carbon bonds are slightly longer and have slightly larger amplitudes of vibration than deuterium–carbon bonds, it might be expected that normal methyl groups will have higher barriers to rotation than deuteromethyl groups, in consideration of the calculations made by Kreevoy and Mason (26) on steric effects and barriers to rotation. Libby suggests (27) "that a further investigation of the dependence of barrier height on isotopic substitution might settle the question as to whether repulsion between hydrogen atoms is the cause of the barrier in ethane". While it is admitted that steric considerations alone certainly grossly oversimplify the problem of barriers to internal rotation, further experimental measurements may well prove postulate (*b*) correct.

On the basis of the two postulates, approximate calculations have been made for a molecule $(CH_3)_2CHX$, where X is a heavy group. For several assumed barrier heights, the contributions to the enthalpy and entropy of activation from the restricted rotation of methyl groups were determined using Pitzer's Tables (28). The use of these tables introduces the additional assumption that the two methyl groups in the isopropyl radical are non-interacting, an assumption which is probably incorrect. However, since the values of the moments of inertia and barrier heights used in the calculation are only approximate, the results contain inherent uncertainties large enough to justify the neglect of any methyl–methyl interaction.

Some results of these calculations are shown in Table III. The top half of this table

TABLE III

Contributions to the secondary β-deuterium isotope effects for the solvolysis of isopropyl compounds in water from internal rotation effects

Barrier to methyl rotation (cal mole^{-1})				
Initial state		Transition state	$(\Delta H_D^* - \Delta H_H^*)_{\text{int rot}}$ per methyl group (cal mole^{-1})	$(\Delta S_D^* - \Delta S_H^*)_{\text{int rot}}$ per methyl group (cal mole^{-1} deg^{-1})
H cpd.	D cpd.	Both cpds.		
3000	3000	0	−65	0.06
3000	3000	600	−29	0.05
3000	3000	1200	−15	0.04
3000	2400	0	−93	−0.26
3000	2400	600	−31	−0.26
3000	2400	1200	−44	−0.26

shows results obtained by the application of postulate (*a*) alone. The entropy of activation difference here is due to the difference in mass between hydrogen and deuterium and is seen to be small and in the direction opposite to that observed in Table II. The bottom half of Table III shows the results obtained by application of both postulates, assuming a 20% difference between the barriers to internal rotation of the normal methyl and deuteromethyl groups. When the figures in the right-hand column, for the entropy of activation difference per methyl group, are multiplied by 2, quite a large part of the observed entropy difference can be explained. At the same time an enthalpy of activation difference is obtained opposite in sign to that springing from a zero-point energy effect.

It is therefore suggested that the major part of the observed isotope effect for the water solvolysis of isopropyl compounds is due to internal rotational effects, and that the most probable cause of the lack of an isotope effect on the enthalpy of activation is a fortuitous cancellation between enthalpy effects arising from zero-point energy sources and internal rotational effects.

EXPERIMENTAL

Isopropyl-β-d_6 alcohol was obtained by the reduction of acetone-d_6 with lithium aluminum hydride using the procedure described by Shiner (29). The crude product was redistilled, boiling point range 83.5–84.5° C. Yields up to 99% were obtained using this procedure. The N.M.R. spectrum of this compound showed that the β-carbon atom was fully deuterated. Treatment of the alcohol with "constant boiling" hydrobromic acid yielded the bromide, boiling point range 60–61° C, in 55% yield after purification.

Isopropyl-β-d_6 methanesulphonate and p-toluenesulphonate were prepared by reaction of the bromide with the appropriate silver sulphonate as previously described for ethyl compounds (30). The sulphonates were purified by distillation under a vacuum.

The protium analogues of the above compounds were prepared by parallel reactions and were used to check the rate constants which had been previously determined in this laboratory (14).

The rate measurements were made conductometrically as described in detail in Part III of this series (31).

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THE CONSTITUTION OF THE HEMICELLULOSE OF APPLE WOOD (MALUS PUMILA L. VAR. GOLDEN TRANSPARENT)[1]

G. G. S. DUTTON AND T. G. MURATA

ABSTRACT

The hemicellulose isolated by direct alkaline extraction of the wood has been shown to be a 4-*O*-methylglucuronoxylan. Methylation data indicate a backbone of approximately 120 D-xylopyranose units linked by β, 1—4 bonds with single units of 4-*O*-methyl-D-glucuronic acid linked by α, 1—2 bonds. The proportion of acid units to xylose is approximately 1:6. Two artifacts were isolated from the hydrolysis products of the methylated polysaccharide: 4-*O*-(2,3-di-*O*-methyl-α-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose and 2,3-di-*O*-methyl-D-lyxose.

Hemicelluloses from many deciduous and coniferous trees have been isolated and studied, and these results have been reviewed (1). The majority of the trees so far investigated have been those of commercial importance for pulping, such as birch, beech, hemlock, and spruce, etc. Certain generalizations on the structure of hemicelluloses have emerged as a result of these studies, the most notable being that the hemicelluloses from coniferous trees have a high mannose content whereas those from deciduous trees are very low in this sugar (1). It was of interest to include other species in these structural investigations and accordingly the hemicelluloses from two members of the Rosaceae family have been studied. The present paper reports the results on *Malus pumila* Mill. (var. Golden Transparent), and the results on *Prunus avium* L. (var. Bing) will be reported later (2).

Finely ground apple wood was extracted with hot alcohol:benzene (1:2) and the air-dried residue was shaken with 0.1 *N* sodium hydroxide. The highly colored extract was discarded and the residue re-extracted with 1 *N* sodium hydroxide according to the method of McDonald (3). The hemicellulose was isolated by pouring the alkaline extract into acidified ethanol and, after being dried by solvent exchange, amounted to 8–12% of the dry wood.

The hydrolysis products of the hemicellulose were resolved on ion exchange resins into a neutral and an acidic fraction. Chromatographic analysis of the former showed it to contain predominantly D-xylose which was characterized as the crystalline sugar. There were also trace amounts of arabinose, galactose, and rhamnose, together with two components having a greater mobility than rhamnose. The identities of these two sugars have not yet been determined. The acidic fraction contained appreciable amounts of an aldobiouronic acid, characterized by conversion to the crystalline methyl 2-*O*-(2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyl)uronate 3,4-di-*O*-acetyl-D-xylopyranoside (4), together with smaller amounts of materials assumed to be free uronic acid and an aldotriouronic acid.

The hemicellulose was methylated, fractionated, and cleaved by methanolysis. After the methyl esters of the acidic components had been saponified with barium hydroxide the mixture of glycosides was resolved into neutral and acidic portions. The acidic fraction was re-esterified with diazomethane and reduced with lithium aluminum hydride with the expectation that the crystalline methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-D-xyloside would be obtained. The sirup did not crystallize, even on

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seeding, although on hydrolysis, 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose were obtained and characterized as their crystalline anilides. Later work (5) has suggested that not all the uronic acid units are terminal and hence the aldobiouronic acid fraction, and thus the derived disaccharide fraction, would not be a single component. This was not apparent in the methylation study reported here.

The neutral sugar glycosides were hydrolyzed and resolved on a cellulose–hydrocellulose column (6). The principal component was 2,3-di-*O*-methyl-D-xylose, with small amounts of 2,3,4-tri-*O*-methyl-D-xylose and 2-*O*- and 3-*O*-methyl-D-xylose. There was also a very small amount of a material judged to be a tri-*O*-methylrhamnose from its chromatographic behavior. Other components isolated were 4-*O*-(2,3-di-*O*-methyl-α-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose, i.e. a methylated xylobiose, and 2,3-di-*O*-methyl-D-lyxose. The former compound has an R_F value of about 0.70 (butanone–water), $[\alpha]_D$ 112° (*c*, 3 in methanol) and a melting point of 83.0–83.5° C. It appears to be identical with the sirupy compound isolated by Geerdes and Smith from methylated flax hemicellulose (7). Since the disaccharide D-xylose-β(1 → 4)-D-xylose is well known and has a negative rotation (8) it may be assumed that its methylated derivative would also be negative. For this reason and also because it has been shown that the acid reversion of D-xylose produces α-linked disaccharides (9) it is assumed that the methylated disaccharide isolated in the present case has the alpha configuration and that it arises by acid reversion from 2,3-di-*O*-methyl-D-xylose. There is, however, the possibility that the native xylan contains a few alpha linkages and that these disaccharides accumulate in the hydrolysis products by analogy with the known greater stability of methyl α-D-glycopyranosides (10). On the other hand, maltose is known to be hydrolyzed faster than cellobiose (10). Until such time as mild hydrolysis of a xylan demonstrates the existence, in nature, of xylose units joined by alpha linkages the reversion theory seems more acceptable. The acid reversion of 2,3-di-*O*-methyl-D-xylose is now being studied.

The second artifact, 2,3-di-*O*-methyl-D-lyxose, caused some trouble. In the column separation of the methylated sugars there was isolated a small amount of a component having an R_F value of 0.40 (butanone–water) and $[\alpha]_D$ −26° in methanol. A compound which was chromatographically identical was also isolated in larger amounts from methylated cherry wood hemicellulose (2). A negative rotation is not recorded for any methyl ethers of D-xylose and two attempts to carry out a hydrobromic acid demethylation (11) led to complete disintegration of the compound. Fortunately the elegant boron trichloride method of demethylation (12) yielded a clean product which was readily identified by chromatography as D-lyxose. The compound 2,3-di-*O*-methyl-D-lyxose has been reported crystalline (13) and has been so obtained by us in another instance (2) but the material isolated here did not crystallize and was too small in amount to convert into a crystalline derivative. The presence of the 2,3-di-*O*-methyl-D-lyxose must be due to a facile epimerization of the D-xylose derivative caused by the alkali present in the barium carbonate used for neutralizing sulphuric acid hydrolyzates. Further work has shown that 2,3-di-*O*-methyl-D-xylose is very readily epimerized by base and these results will be reported later. A recheck of the hydrolyzate of the original polysaccharide failed to show the presence of any lyxose. Epimerization of methylated sugars from polysaccharides has not previously been recorded, although it is well known in the case of monosaccharides and has also been described in the case of aldobiouronic acids (14, 15).

A sample of the neutral methylated sugars was chromatographed, and their ratio estimated by the phenol – sulphuric acid method (16). These values together with that

obtained gravimetrically for the aldobiouronic acid gave a mole ratio of 1:97:21:19 for tri-, di-, mono-*O*-methyl pentoses, and uronic acid respectively. The slight excess of mono-*O*-methyl pentose over that required by the uronic acid might indicate a small degree of branching, but in view of the observations of Croon and Timell this seems unlikely (17). These results therefore indicate that apple wood hemicellulose has a chain length of approximately 120 D-xylose units and a xylose:uronic acid ratio of 6:1. It would thus appear that this hemicellulose is similar in structure to other wood hemicelluloses except that the proportion of uronic acid is higher than has been found for the majority of deciduous trees so far examined. This ratio is usually about 10 or 11 to 1 (1) although white elm has been found to have a ratio of 7:1 (18). In general it is characteristic of coniferous woods to have a high uronic acid content, i.e. 5 or 6 to 1.

EXPERIMENTAL

The following solvent systems (v/v) were used: (A) ethyl acetate:acetic acid:formic acid:water, 18:3:1:4; (B) 1-butanol:ethanol:water:ammonia, 40:11:19:1; (C) butanone–water azeotrope; (D) 1-butanol:acetone:water, 5:4:1. With solvent system D, phosphate-impregnated papers, which were prepared by dipping papers into a disodium hydrogen phosphate and potassium dihydrogen phosphate buffer (pH 5), were used (19). Electrophoresis was carried out using 0.05 *M* sodium borate (20). Evaporations were carried out *in vacuo* at 40° C and rotations were recorded at 22±2° C.

Isolation of Apple Wood Hemicellulose

Sawdust (200 g) was extracted with hot alcohol–benzene (1:2) for 4 hours and air dried. The sawdust was suspended in sodium hydroxide (0.1 *N*, 1.8 l.) for 24 hours at room temperature, after which the mixture was filtered and the filtrate discarded. The residue was washed with water and then extracted with sodium hydroxide (1 *N*, 1.8 l.) with shaking, for 48 hours, at room temperature. The suspension was filtered and the residue washed with water to give a total volume of 2 l. The hemicellulose was isolated by pouring the extract into cold ethanol containing acetic acid. The material thus obtained was dried by solvent exchange and a typical preparation had $[\alpha]_D$ −62° (*c*, 1.2 in 8% NaOH) and an ash content of 4.7%. The yield was 10–12 g of hemicellulose per 100 g of sawdust.

Hydrolysis of the Hemicellulose

Hemicellulose (214 mg) in sulphuric acid (1 *N*) was heated on a steam bath for 9 hours during which time the rotation changed from $[\alpha]_D$ −62° to +41°. Sulphuric acid was neutralized with barium carbonate and the solution was passed through Amberlite IR 120 and Duolite A4 resins. Evaporation of the neutral eluate yielded a sirup (144 mg), which was examined in solvents A, B, C, and D. In each system a large spot corresponding to xylose was observed, in solvent D traces of arabinose, rhamnose, and galactose were observed, and in solvent C two additional components with R_F 0.17 and 0.30 were detected.

The acidic components were eluted from the anion resin with sodium hydroxide (2 *N*, 5 ml), and the free acids were liberated by passage through fresh cation resin. Evaporation yielded a sirup (24.5 mg) which, in solvent A, showed three components having R_{xylose} 1.2, 1.0, and 0.69. These correspond to uronic acid, aldobiouronic acid, and aldotriouronic acid (21).

In a similar way, approximately 30 g of hemicellulose was hydrolyzed to yield 22.14 g of neutral sugars and 5.84 g of acids. The sirup containing the neutral sugars crystallized

spontaneously, and the D-xylose after recrystallization from methanol–water had m.p. and mixed m.p. 143–145° C and $[\alpha]_D$ 17.7° (*c*, 1.69 in water, equil.). The mother liquor contained a component chromatographically and electrophoretically indistinguishable from rhamnose and also two compounds with R_F values 0.17 and 0.30 in solvent C.

A portion of the acid sirup was streaked on Whatman 3MM paper and irrigated with solvent A for 16 hours. The section containing the component with R_x 1.0 was eluted with methanol and this component was characterized as methyl 2-*O*-methyl(2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyl)uronate 3,4-di-*O*-acetyl-D-xylopyranoside according to the method of Timell (4); m.p. 191–193° C, undepressed by an authentic sample.

Methylation and Methanolysis of the Hemicellulose

The hemicellulose was methylated with methyl sulphate and sodium hydroxide five times and then three times with methyl iodide and silver oxide. After these treatments there was no hydroxyl band in the I.R. spectrum and the methoxyl content was 38.7%. Fractional precipitation of a chloroform solution with petroleum ether (30–60°) gave a fraction having $[\alpha]_D$ −57.8° (chloroform) and methoxyl 39.1%. A portion of this material (2.02 g) was refluxed for 7 hours in 3% methanolic hydrogen chloride, and after neutralization (Ag_2CO_3) and evaporation there was obtained a dark sirup of the methyl glycosides (2.13 g). This sirup was dissolved in saturated barium hydroxide (25 ml) and heated at 60° for 2 hours. The aqueous solution was continuously extracted with petroleum ether (30–60°) for 20 hours and was then resolved into neutral and acidic fractions by Amberlite IR 120 and Duolite A4 resins in the usual way. The petroleum ether extract was added to the sirup from the neutral eluate and re-evaporation gave the methyl glycosides (1.52 g). The acidic component amounted to 475 mg.

Identification of the Acidic Component

The acidic component was dissolved in methanol and treated with diazomethane. The resulting ester was dissolved in dry tetrahydrofuran (75 ml), and a solution of lithium aluminum hydride (500 mg) in the same solvent (20 ml) was slowly added, with stirring, at room temperature. Reduction was completed by refluxing for 1 hour, and the product was isolated by acetylation as previously described (22). The colorless sirup (344 mg) failed to crystallize even on seeding. Accordingly a portion of the disaccharide (186 mg) was dissolved in methanol (10 ml) containing acetyl chloride (0.5 ml) and refluxed for 5 hours. The mixture was neutralized (Ag_2CO_3), filtered, and evaporated to give a mixture of glycosides (202 mg) which were then hydrolyzed by sulphuric acid (1 *N*, 5 ml) on the steam bath for 7 hours. The solution was deionized with Duolite A4 and after concentration, chromatographic and electrophoretic examination revealed components identical with 2,3,4,-tri-*O*-methyl-D-glucose, 3-*O*-methyl-D-xylose, and a trace of what was assumed to be incompletely hydrolyzed material.

The mixture of sugars (126 mg) was separated on a cellulose–hydrocellulose column (6) using butanone–water azeotrope (36 ml per hour) and fractions were collected at 20-minute intervals. Concentration of the contents of tubes 11–17 gave a sirup (37 mg), $[\alpha]_D$ 73° (*c*, 0.7 in ethyl acetate), chromatographically identical with 2,3,4-tri-*O*-methyl-D-glucose. This was confirmed by the preparation of 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine, which had m.p. 141–143° C on recrystallization from ethyl acetate – petroleum ether (lit. value 145–146° C (23)).

Concentration of the contents of tubes 60–80 gave a sirup (24 mg) electrophoretically identical with 3-*O*-methyl-D-xylose. The derived 3-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. 133–134° C (lit. value m.p. 137° C (24)).

Identification of the Neutral Sugars

The methyl glycosides (1.36 g) were hydrolyzed with sulphuric acid (1 *N*, 60 ml) by heating the mixture on the steam bath for 8 hours. Neutralization ($BaCO_3$) and evaporation yielded a sirup (1.16 g), part of which (1.04 g) was separated on a cellulose–hydrocellulose column as before. One hundred fractions were collected at 10-minute intervals and a further 100 fractions at 30-minute intervals. The results are shown in Table I.

TABLE I

Separation of methylated sugars of apple wood hemicellulose

Component	Tube number	Weight, mg	Identity
1	6–9	17.0	Tri-*O*-methylrhamnose
	10		1+2
2	11–13	21.9	2,3,4-Tri-*O*-methyl-D-xylose
	14–15	14.7	2+3
3	16–22	56.0	Methylated xylobiose
	23–31	32.6	3+4
4	32–53	637.6	2,3-Di-*O*-methyl-D-xylose
	54–59	22.0	4+5
5	60–75	23.0	2,3-Di-*O*-methyl-D-lyxose
6	109–113	15.6	2- and 3-*O*-Methyl-D-xylose
7	160–185	8.6	D-Xylose
		849	

NOTE: Recovery 82%.

Component 1.—This had R_F 0.86 and gave a characteristic pale yellow color with the *p*-anisidine spray. It was thus tentatively identified as a tri-*O*-methylrhamnose.

Component 2.—This was chromatographically identical with 2,3,4-tri-*O*-methyl-D-xylose. The rotation of the sirup, $[\alpha]_D$ ca. 6° (*c*, 0.4 in methanol), indicated that it was not pure and attempts to crystallize the sirup and prepare the crystalline *N*-phenylglycosylamine both failed.

Component 3.—This fraction had R_F 0.71 and $[\alpha]_D$ 112° (*c*, 3 in methanol). On evaporation of the solvent it readily crystallized to long, feathery needles. All attempts to recrystallize the solid failed but it was readily recovered in crystalline form by allowing the solvent to evaporate; m.p. 83.0–83.5° C. Hydrolysis with hot sulphuric acid (1 *N*) gave a compound chromatographically identical with 2,3-di-*O*-methyl-D-xylose.

Component 4.—This material had R_F 0.50 and was the major component. It was identified as 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m.p. 122–123° C, on recrystallization from ethyl acetate – petroleum ether (lit. m.p. 121–123° C (25)).

Component 5.—This fraction had R_F 0.40 and was present in only small amount. It had $[\alpha]_D$ ca. −26° in methanol and was chromatographically identical with the material characterized as 2,3-di-*O*-methyl-D-lyxose isolated from cherry wood hemicellulose (2). This was confirmed as follows. A portion of the unknown sugar (10 mg) was dissolved in dry dichloromethane (3 ml) and the solution cooled to −78°. Boron trichloride (1–2 g) was distilled into this solution and the temperature maintained at −78° for 30 minutes. The solution was allowed to come to room temperature and to evaporate overnight. Electrophoresis and chromatography (solvent D) of the residue dissolved in aqueous methanol indicated the presence of lyxose.

Component 6.—This was readily shown by electrophoresis to be a mixture of 2-*O*- and 3-*O*-methyl-D-xylose.

Component 7.—This was chromatographically identical with xylose.

Quantitative Analysis of Neutral Sugars

A portion of the neutral sugars was separated on Whatman No. 1 paper using butanone–water azeotrope. The zones corresponding to tri-, di-, and mono-*O*-methylpentoses were separately eluted with water and the concentration of the sugars determined by the phenol – sulphuric acid method (16). The ratios thus determined were 1:97:2. Standard curves were prepared from 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-xylose.

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THE CHEMISTRY OF ALUMINUM–NITROGEN COMPOUNDS

I. REACTIONS OF TRIMETHYLALUMINUM AND TRIMETHYLALUMINUM TRIMETHYLAMINE WITH SEVERAL METHYLHYDRAZINES[1]

NEIL R. FETTER AND BODO BARTOCHA

ABSTRACT

The reactions of trimethylaluminum and trimethylaluminum trimethylamine with various methylhydrazines have produced a number of complexes and polymers: with methylhydrazine, both Me_3Al and $Me_3Al{:}NMe_3$ produce the polymer $[—MeAlNHNMe—]_n$; with 1,2-dimethylhydrazine, both produce the polymer $[—Al(MeNNMe)_{1.5}—]_n$; with 1,1-dimethylhydrazine, Me_3Al produces $[Me_2Al—NHNMe_2]_2$ and $Me_3Al{:}NMe_3$ produces the unusual complex $Me_2Al(NHNMe_2)(NH_2NMe_2)$; with trimethylhydrazine, Me_3Al produces $[Me_2Al—NMeNMe_2]_2$ and $Me_3Al{:}NMe_3$ produces an isomer with the same formula but an as-yet-uncharacterized structure. In addition, the intermediate $Me_3Al{:}NHMeNMe_2$ has been isolated from the reaction of either Me_3Al or $Me_3Al{:}NMe_3$ with trimethylhydrazine. With tetramethylhydrazine, Me_3Al produces $Me_2Al{:}NMe_2NMe_2$. The formation of the complexes with covalent aluminum–nitrogen bonds is specific and methane is the only gas eliminated during these reactions.

INTRODUCTION

Until recently, only a few molecular addition compounds of aluminum with ligands of group V had been reported (1). In some of the earliest work, Brown and Davidson (2) described methylaluminum–methylamine complexes. Later, Wiberg and May (3) reported some reactions of aluminum hydride with amines and ammonia, an area of interest recently extended by studies of Ruff and Hawthorne (4, 5). Bonitz (6) isolated a triethylaluminum isoquinoline complex, and Ziegler, Gellert, and Neumann (7) base some analytical procedures on the reaction of alkylaluminum hydrides with amines. The compound $Et_2Al—NMe_2$ was reported in the patent literature (8), and a note on a new aluminum hydride adduct appeared in print (9).

Most of the compounds described in these previous publications contain a covalent aluminum–nitrogen bond which is formed by one of two general reactions:

(1) elimination of a hydrocarbon when an aluminum trialkyl reacts with a secondary or primary amine:

$$R_3Al + NHR_2' \rightarrow [R_3Al{:}NHR_2'] \rightarrow R_2Al—NR_2' + RH;$$

(2) elimination of hydrogen when an alkylaluminum hydride or aluminum hydride reacts with ammonia or a primary or secondary amine:

$$R_2AlH + NH_3 \rightarrow [R_2AlH{:}NH_3] \xrightarrow{\Delta} R_2Al—NH_2 + H_2,$$

$$R_2AlH + NHR_2' \rightarrow [R_2AlH{:}NHR_2'] \xrightarrow{\Delta} R_2Al—NR_2' + H_2.$$

In both cases a stable covalent bond is formed via an intermediate with a dative bond, and in several instances the materials seem to react further to form polymeric substances (10).

We were interested in the interactions of hydrazines with aluminum alkyls or aluminum hydride, and, in particular, of those hydrazines which carry both functional groups, namely, the N—H and N—C bonds. In this paper we report the reactions of trimethylaluminum and trimethylaluminum trimethylamine with methylhydrazines. Several new compounds with covalent or dative Al—N bonds were synthesized, and their physical constants determined.

[1]*Manuscript received April 7, 1961.*
Contribution from U.S. Naval Ordnance Laboratory, Corona, Calif., U.S.A.

EXPERIMENTAL

Apparatus

Most of the reactions were carried out in a conventional high-vacuum system. The hydrazine was transferred *in vacuo* to a cold trap (−196° C) containing the Me_3Al or $Me_3Al{:}NMe_3$ and any necessary solvent, such as pentane. After the mixtures had reached room temperature or had been heated so that the reaction would be completed, the solvent was pumped off and, if possible, the product was purified by sublimation.

In a few cases, use of the vacuum technique resulted in explosions, and the reactions were therefore carried out at atmospheric pressure under a nitrogen atmosphere. The hydrazine was added slowly to a cooled (−10° C) pentane solution of Me_3Al or $Me_3Al{:}NMe_3$. After the addition had been completed and the reaction mixture had reached room temperature, the solvent was removed in vacuum.

Molecular Weights

Molecular-weight determinations were conducted in a conventional freezing-point apparatus with cyclohexane as the solvent. The inner vessel was designed so that samples and solvent could be weighed in a dry box.

Reagents

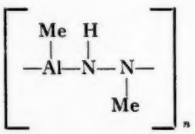
Methylhydrazine (Commercial Solvents Corporation), 1,1-dimethylhydrazine (Olin Mathieson Corporation), 1,2-dimethylhydrazine (Metalectro Corporation), and tetramethylhydrazine (Metalectro Corporation) were distilled over calcium hydride before use. The trimethylhydrazine was prepared by the method of Class, Aston, and Oakwood (11). Trimethylaluminum was purchased from the Ethyl Corporation of Baton Rouge, Louisiana, and vacuum-distilled before use. The preparation of trimethylaluminum trimethylamine was based on a procedure described by Brown and Davidson (2). All solvents were reagent grade, and all were distilled over calcium hydride before use.

Analysis

The evolved gases were measured by fractionating the mixtures through a series of traps into a calibrated volume and removing the uncondensable material with a Toepler pump. Aluminum was determined as Al_2O_3 after the sample had been decomposed with methanol and ashed. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside 77, New York.

Reaction of Trimethylaluminum Trimethylamine with Methylhydrazine

The atmospheric method was used for the reaction of $Me_3Al{:}NMe_3$ with H_2NNHMe. After 1.53 g (11.7 mmoles) of $Me_3Al{:}NMe_3$ and 0.602 g (13.1 mmoles) of H_2NNHMe had reacted and the solvent had been removed, a powdery white solid remained. This material could not be sublimed and was insoluble in hydrocarbons. It did not have a sharp melting point but decomposed slowly when heated to 200° C. Elemental analysis gave results which agree with the empirical formula $C_2H_7N_2Al$. Calc.: Al, 31.34%; C, 27.90%; H, 8.20%; N, 32.55%. Found: Al, 30.40%; C, 28.18%; H, 8.05%; N, 30.37%. From this formula, the following structure may be written:



The equation for the reaction is

$$n\mathrm{Me_3Al{:}NMe_3} + n\mathrm{H_2NNHMe} \rightarrow [\mathrm{—MeAlNHNMe—}]_n + 2n\mathrm{CH_4} + n\mathrm{NMe_3}.$$

Hydrolysis of 0.243 g (2.83 mmoles) of the product yielded 0.0338 g (2.11 mmoles) of methane. The molar ratio of CH_4 to $[\mathrm{—MeAlNHNMe—}]_n$ is 0.775.

Reaction of Trimethylaluminum with Methylhydrazine

By the procedure described above, the reaction of Me_3Al with methylhydrazine produces a material whose physical properties are the same as those of the $Me_3Al{:}NMe_3$–methylhydrazine polymer. An aluminum assay gave 30.55% Al (calc.: 31.34%), and the infrared spectra of the two materials were identical. The reaction may thus be written:

$$n\mathrm{Me_3Al} + n\mathrm{H_2NNHMe} \rightarrow [\mathrm{—MeAlNHNMe—}]_n + 2n\mathrm{CH_4}.$$

Reaction of Trimethylaluminum with 1,1-Dimethylhydrazine

By the vacuum method, the direct reaction of 0.680 g (9.45 mmoles) of Me_3Al with 0.632 g (10.5 mmoles) of H_2NNMe_2 gave a crystalline solid (m.p. 77–78.5° C)* and 0.143 g (8.45 mmoles) of methane. The molar ratio of CH_4 to Me_3Al is 0.945. Sublimed once, at 60° and 0.005 mm Hg, the reaction product was analyzed, and the results were found to agree with the empirical formula $C_4H_{13}N_2Al$. Calc.: Al, 23.23%; C, 41.36%; H, 11.28%; N, 24.13%. Found: Al, 23.82%; C, 41.12%; H, 11.04%; N, 24.21%. Two molecular-weight values of 238 and 240 indicate that this compound is dimeric (calc.: 230.2). The equation for the reaction is

$$2\mathrm{Me_3Al} + 2\mathrm{H_2NNMe_2} \rightarrow [\mathrm{Me_2Al—NHNMe_2}]_2 + 2\mathrm{CH_4}.$$

Reaction of Trimethylaluminum Trimethylamine with 1,1-Dimethylhydrazine

Again by the vacuum method, direct reaction of 1.63 g (10.4 mmoles) of $Me_3Al{:}NMe_3$ with 1.62 g (27.1 mmoles) of H_2NNMe_2 yielded a white crystalline solid (m.p. 81–82.0° C), 0.141 g (8.86 mmoles) of methane, and 0.451 g (7.65 mmoles) of trimethylamine. The molar ratio of CH_4 to $Me_3Al{:}NMe_3$ is 0.852, and the molar ratio of NMe_3 to $Me_3Al{:}NMe_3$ is 0.746. Elemental analysis of the product, which had been purified by sublimation at 30° C and 0.005 mm Hg, gave results which correspond to the percentages calculated for the empirical formula $C_6H_{21}N_4Al$. Calc.: Al, 15.30%; C, 40.89%; H, 12.01%; N, 31.78%. Found: Al, 15.95%; C, 40.60%; H, 11.77%; N, 31.58%. These data and a molecular-weight determination of 179 (calc.: 176.1) suggest the structural formula

$$\begin{array}{c} \mathrm{NHNMe_2} \\ | \\ \mathrm{Me—Al{:}NH_2NMe_2.} \\ | \\ \mathrm{Me} \end{array}$$

Hydrolysis of 0.275 g (2.08 mmoles) of the complex produced 0.0685 g (4.28 mmoles) of methane; thus, the expected 2:1 molar ratio of methane to complex was obtained. The reactions of Me_3Al and $Me_3Al{:}NMe_3$ with 1,1-dimethylhydrazine are not influenced by the ratios of starting materials. An experiment with a molar ratio of 1:1 for H_2NNMe_2 and $Me_3Al{:}NMe_3$ yielded only $Me_2Al(NHNMe_2)(H_2NNMe_2)$ and an experiment with a starting molar ratio of 1.5:1 for H_2NNMe_2 and Me_3Al produced only $[\mathrm{Me_2Al—NHNMe_2}]_2$.

Reaction of Trimethylaluminum Trimethylamine with 1,2-Dimethylhydrazine

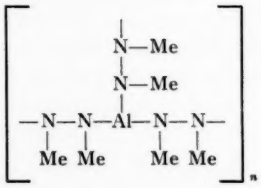
By the vacuum method, 0.812 g (6.20 mmoles) of $Me_3Al{:}NMe_3$ was treated with 0.530 g (8.83 mmoles) of NHMeNHMe at room temperature for 18 hours. At the end of this

*All melting points reported are uncorrected.

period a white precipitate had formed, and 0.293 g (18.3 mmoles) of methane and 0.427 g (5.77 mmoles) of NMe_3 had been recovered. The molar ratio of recovered CH_4 to starting $Me_3Al{:}NMe_3$ is 2.96, and the molar ratio of NMe_3 to $Me_3Al{:}NMe_3$ is 0.932. The product was insoluble in hydrocarbons, did not have a sharp melting point, and could not be sublimed; however, on elemental analysis, the crude product gave results which agree with the formula $C_3H_9N_3Al$. Calc.: Al, 23.65%; C, 31.58%; H, 7.94%. Found: Al, 24.09%; C, 30.34%; H, 7.94%. From these data, the following reaction may be formulated:

$$n\text{Me}_3\text{Al:NMe}_3 + 1.5n\text{NHMeNHMe} \rightarrow [\text{—Al(MeNNMe)}_{1.5}\text{—}]_n + 3n\text{CH}_4 + n\text{NMe}_3.$$

The reaction product probably is a completely cross-linked polymer, with each aluminum atom incorporating one half of three [—MeNNMe—] units; hence the structural formula may be written as follows:



Hydrolysis of this material produces no methane, as expected, and the infrared spectrum shows no N—H bond in the 3.05-μ region.

Trimethylaluminum reacts with 1,2-dimethylhydrazine to produce the same polymer.

Reaction of Trimethylaluminum with Trimethylhydrazine

In all the reactions discussed above, the elimination of methane accompanied the complex formation. However, in the reaction of Me_3Al with $HNMeNMe_2$, the adduct intermediate was isolated and then converted into the covalent structure with elimination of methane. The intermediate was fairly stable; in fact, heating the reaction mixture for 20 hours at 60° C was required to effect the conversion.

By the vacuum procedure, 3.53 g (49.1 mmoles) of Me_3Al was treated with 9.61 g (130 mmoles) of $HNMeNMe_2$ at room temperature. From the liquid residue remaining after removal of the excess trimethylamine, a volatile, white crystalline solid (m.p. 80.0–83.0° C) was isolated by evaporation at 25° C onto a cold finger maintained at −15° C. The elemental analysis corresponds to the values for the formula $C_6H_{19}N_2Al$. Calc.: Al, 18.45%; C, 49.29%; H, 13.10%; N, 19.16%. Found: Al, 18.58%; C, 50.14%; H, 12.60%; N, 18.92%. A molecular-weight measurement gave the value 142.0 (calc.: 146.2). The initial reaction may thus be written:

$$\text{Me}_3\text{Al} + \text{HNMeNMe}_2 \rightarrow \text{Me}_3\text{Al:NHMeNMe}_2.$$

I

Compound I was then heated for 20 hours at 60° C and resublimed. A white crystalline solid (m.p. 125.0–126.5° C) was obtained and analyzed. Calc. for $C_5H_{15}N_2Al$: Al, 20.73%; C, 46.13%; H, 11.61%; N, 21.52%. Found: Al, 20.34%; C, 44.82%; H, 11.77%; N, 22.82%. A molecular-weight determination gave the value 258.0 (calc.: 260.4). The elimination reaction may then be written as follows:

$$2\text{Me}_3\text{Al:NHMeNMe}_2 \xrightarrow{60^\circ\text{ C}} [\text{Me}_2\text{Al—NMeNMe}_2]_2 + 2\text{CH}_4.$$

II

Compound II was also synthesized directly from Me_3Al and trimethylhydrazine by heating together 1.24 g (17.3 mmoles) of Me_3Al and 1.26 g (17.1 mmoles) of $HNMeNMe_2$ to 55° C for 20 hours. A crystalline solid (m.p. 125.0–126.5° C) and 0.270 g (16.8 mmoles) of methane were produced. Calc.: Al, 20.73%; C, 46.13%; H, 11.61%; N, 21.52%. Found: Al, 20.42%; C, 45.59%; H, 11.52%; N, 23.49%.

Reaction of Trimethylaluminum Trimethylamine with Trimethylhydrazine

In its initial step, this reaction is similar to that between Me_3Al and $HNMeNMe_2$ except that NMe_3 is displaced during the formation of the intermediate:

$$Me_3Al{:}NMe_3 + HNMeNMe_2 \rightarrow Me_3Al{:}NHMeNMe_2 + NMe_3.$$

In vacuo, 1.34 g (10.2 mmoles) of $Me_3Al{:}NMe_3$ was heated at 60° C with 0.813 g (11.0 mmoles) $HNMeNMe_2$ until NMe_3 evolution ceased (20 hours). Elemental analysis of this sublimed complex (m.p. 80.0–83.0° C) agreed with the formula for I. Calc.: Al, 18.45%; C, 49.29%; H, 13.10%; N, 19.16%. Found: Al, 18.27%; C, 50.99%; H, 13.33%; N, 18.57%.

However, in another experiment 1.96 g (14.9 mmoles) of $Me_3Al{:}NMe_3$ and 2.31 g (31.1 mmoles) of $HNMeNMe_2$ were heated at 75° C for 2 days. When gas evolution ceased, a clear liquid remained, and 0.192 g (12.0 mmoles) of CH_4 and 0.576 g (9.77 mmoles) of NMe_3 had been recovered. The liquid was vacuum-distilled at 25° C into a −30° C trap, and elemental analysis of the distillate (m.p. 13.5–15.0° C) gave results that correspond to the values for II. Calc.: Al, 20.73%; C, 46.13%; H, 11.61%; N, 21.52%. Found: Al, 21.94%; C, 44.09%; H, 11.63%; N, 23.11%. The reaction may be written as follows:

$$Me_3Al{:}NMe_3 + HNMeNMe_2 \xrightarrow[\text{excess } HNMeNMe_2]{75^\circ\ C} C_5H_{15}N_2Al + CH_4 + NMe_3.$$

Two molecular-weight measurements gave the values 189 and 195 (calc. for the monomer: 130.2; for the dimer: 260.4). Hydrolysis of 0.229 g (17.6 mmoles) produced 0.0483 g (30.2 mmoles) of methane; hence, the molar ratio of CH_4 to $C_5H_{15}N_2Al$ is 1.72. These data indicate that this complex is not compound II, but that it does contain an Me_2Al group and its empirical formula is the same as that for II. The molcular-weight data suggest a complex which is only partially associated in cyclohexane; however, the data are insufficient to indicate structural formula at this time.

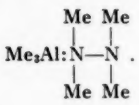
Two attempts were made to convert compound II to the liquid complex described above. In the first attempt, 0.5 g of compound II was heated at 75° C for 48 hours followed by 120° C for 2 hours. The material remained unchanged. In the second attempt, 0.5 g of II was heated at 75° C for 48 hours with 1.0 g of trimethylhydrazine. After removal of the trimethylhydrazine an attempt to vacuum-distill the liquid residue at 90° C under 0.001 mm Hg pressure failed. The compound would not distill. The infrared spectrum of the liquid indicated it was neither II nor the material obtained above.

It should be noted that the temperature employed to prepare $Me_3Al{:}HNMeNMe_2$ from $Me_3Al{:}NMe_3$ and trimethylhydrazine (60° C) is also that used to convert $Me_3Al{:}HNMeNMe_2$ into $Me_2Al—NMeNMe_2$ when the former complex is prepared from Me_3Al and trimethylhydrazine. The authors have no explanation for this anomaly, but it must be presented as the condition under which these reactions occurred.

Reaction of Trimethylaluminum with Tetramethylhydrazine

By the vacuum method, 1.10 g (15.3 mmoles) of Me_3Al was treated with 1.50 g (17.0 mmoles) of Me_2NNMe_2 for 2 hours at room temperature. No gas evolution was observed

during this period, and a white crystalline solid formed in the reaction vessel. The material was sublimed at 60° C (m.p. 65.5–66.0° C), and elemental analysis of the sublimate agreed with the values calculated for the 1:1 complex:



Calc.: Al, 16.84%; C, 52.47%; H, 13.21%; N, 17.48%. Found: Al, 16.87%; C, 52.48%; H, 13.03%; N, 17.04%. A molecular-weight measurement gave the value 164 (calc.: 160.3); hence, the following reaction may be written:

$$Me_3Al + Me_2NNMe_2 \xrightarrow{25^\circ C} Me_3Al{:}NMe_2NMe_2.$$

All of the compounds described above hydrolyze rapidly in methanol, ethanol, and 1,4-dioxane and react vigorously with water. They decompose in air without inflaming and they react slowly with dry air. The polymeric compounds are somewhat less sensitive to moisture and oxygen. Yields of purified products were approximately 65–70 mole% of the initial quantity of Me_3Al or $Me_3Al{:}NMe_3$.

DISCUSSION

The reactions described above occur by two sequential steps. The first is the formation of a dative-bonded complex of trimethylaluminum and the hydrazine:

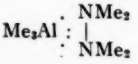
$$Me_3Al + R_2NNR_2 \rightarrow Me_3Al{:}R_2NNR_2. \qquad [1]$$

The second is the elimination of methane to form a complex with a covalent aluminum–nitrogen bond:

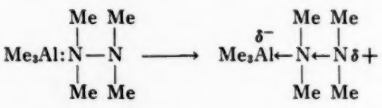
$$Me_3Al{:}RHNNR_2 \rightarrow Me_2Al{-}NRNR_2 + CH_4. \qquad [2]$$

The structure of the dative-bonded complexes cannot be determined from the data available, but it should be noted that in the two cases wherein the complexes could be isolated, i.e., the reactions of trimethylhydrazine and tetramethylhydrazine with trimethylaluminum, only 1:1 adducts are formed. This fact suggests a structure which prohibits further complexing of another Me_3Al molecule to the other nitrogen atom of the hydrazine.

A complex involving a pentaco-ordinated aluminum atom:



or the charge distribution on the structure with a tetraco-ordinated aluminum atom:



would inhibit further addition; hence one of these two formulas may represent the configuration of the dative-bonded adducts.

The elimination of methane during the formation of the covalent-bonded complexes

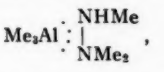
is a striking feature of *all* the reactions in which such elimination occurs. The absence of ethane which would be liberated if the following mechanism:

$$Me_3Al + H_2NNMe_2 \rightarrow Me_2AlNMeNH_2 + C_2H_6, \qquad [3]$$

was followed indicates that in unsymmetrical hydrazines, the nitrogen atom with the largest number of N—H bonds forms the covalent bond with aluminum:

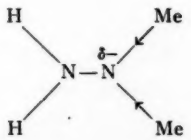
$$Me_3Al{:}MeHNNMe_2 \rightarrow Me_2Al{-}NMeNMe_2 + CH_4.$$

The specificity of this reaction may be the combined effect of steric and electronic factors. For example, with unsymmetrical hydrazines, if one assumes the formation of a tetraco-ordinated intermediate, the Me_3Al may bond with the least sterically hindered nitrogen atom, namely, the one with the greatest number of N—H bonds and the subsequent methane elimination occurs from these adjacent aluminum and nitrogen atoms. If one postulates the pentaco-ordinated aluminum complex:



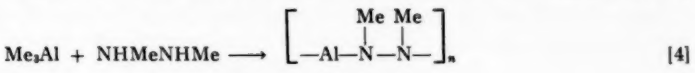
the aluminum atom may be forced into closer proximity to the nitrogen atom with N—H bonds, and covalent bonding with aluminum would tend to occur here.

The classical theory of inductive effect would assign to the nitrogen atom of an unsymmetrical hydrazine with the greatest number of methyl groups the greater negative charge:

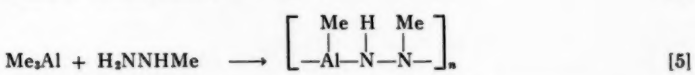


One would thus expect a Lewis acid such as Me_3Al to form a dative bond with this nitrogen atom. The formation of the covalent bond should produce ethane, however (eq. [3]). Since none is observed, this hypothesis is not tenable. If electronic effects influence the formation of the covalent Al—N bond, then they are of an order that cannot be inferred from the available data.

Analysis of the reaction products obtained from trimethylaluminum and either methylhydrazine (eq. [4]) or 1,2-dimethylhydrazine (eq. [5]) shows that both nitrogen atoms react with equal probability. It has been observed from the evidence available (7, 8)

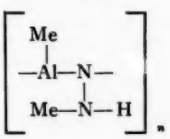


[4]



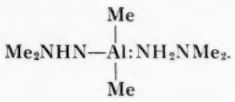
[5]

that no more than one aluminum trialkyl molecule will add to the nitrogen atom of H_2NMe; hence, the alternative structural formula:

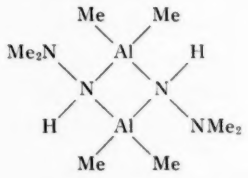


for the product of reaction [4] seems unlikely. Even when a large excess of trimethylaluminum is present and even under vigorous reaction conditions (i.e., heating for a prolonged period of time), we also found no indication that an $—NH_2$ group would react more than once with the trimethylaluminum.

A very interesting compound is formed in the reaction between 1,1-dimethylhydrazine with trimethylaluminum trimethylamine. Brown and Davidson (2) report that $Me_2Al—NMe_2$ does not react further with diethyl ether or trimethylamine to form a dative-bonded complex; however, in the case of the hydrazine reaction, a compound was isolated which appeared to contain both covalent and dative Al—N bonds:



Our molecular-weight determinations have shown that at least two of the compounds are dimeric: $[Me_2Al—NHNMe_2]_2$ and $[Me_2Al—NMeNMe_2]_2$. It has been shown (12, 13) that similar boron compounds associate through nitrogen bridges; hence, it is reasonable to assume that the dimeric compounds which we prepared also associate through Al—N bridges:



In conclusion, it can be said that, in general, hydrazines react with trimethylaluminum in the same manner as do primary or secondary amines, but the reactions of methylhydrazines and trimethylaluminum trimethylamine have produced, in some cases, compounds of unusual character. We believe that additional insight into the nature of these and similar reactions will be provided by work currently in progress at this laboratory.

ACKNOWLEDGMENTS

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VIBRATIONAL SPECTRA OF PYRIDINIUM SALTS[1]

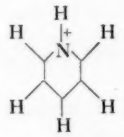
DENYS COOK

ABSTRACT

The vibrational spectrum of the pyridinium ion, $C_5H_5NH^+$, and the N-deuterated species has been studied in several pyridinium salts. By comparison with benzene and deuterobenzene (with which the pyridinium ion is isoelectronic) a fairly complete assignment has been made. The N—H bonds, which are hydrogen bonded to the anions, have stretching vibrations which show systematic variations depending on the nature of the anion. The N—H in-plane deformation vibrations show little variation while the out-of-plane deformation vibrations, some of which do vary, are probably affected, in addition, by other factors.

INTRODUCTION

Pyridine forms salts with many strong mineral and organic acids. In these salts it is assumed that complete proton transfer takes place between the acid and the pyridine molecule, giving the pyridinium ion I and an anion.



I

Little spectroscopic data on such salts is available in the published literature.* Greenwood and Wade (1) assigned a few bands from their published spectra of $C_5H_5NH^+Cl^-$ and $C_5H_5NH^+BCl_4^-$, while Kynaston and co-workers (2) listed all the bands in the latter salt. Spinner published a list of frequencies in the infrared and Raman spectrum (aqueous solution) of $C_5H_5NH^+Cl^-$ (3) and made a few assignments.

In these papers emphasis was placed on similarities between these salts and C_5H_5N. It is believed that a more pertinent and helpful comparison is between the pyridinium ion and C_6H_6. These molecules contain the same number of atoms, are isoelectronic, and, apart from the mass and electronegativity of the nitrogen atom, would be identical. This physical similarity breaks down, however, when the symmetry of the two molecules is compared. C_6H_6 has D_{6h}, and $C_5H_5NH^+$ is expected to have C_{2v} symmetry. Thus C_6H_6 has only four (strictly) infrared-active fundamentals and seven Raman fundamentals. $C_5H_5NH^+$ will have 30 Raman-active fundamentals and 27 infrared-active fundamentals. (The selection rules are shown in Table I.) The comparison between C_6H_5D and $C_5H_5ND^+$, however, reveals a much closer similarity, since both molecules now have C_{2v} symmetry, with identical selection rules.

[1]*Manuscript received May 26, 1961.*

Contribution No. 39 from the Exploratory Research Laboratory, Dow Chemical of Canada, Limited, Sarnia, Ontario. This work was presented, in part, at the Seventh Ottawa Symposium on Applied Spectroscopy, October 5, 1960.

**As this paper was being prepared, a partial listing of the frequencies in some pyridinium salts was published (R. H. Nuttall, D. W. A. Sharpe, and T. C. Waddington. J. Chem. Soc. 4965 (1960)), but not in a complete enough state to warrant comparison at this time.*

TABLE I

Selection rules for the pyridinium ion (symmetry C_{2v})

Class	Symmetry elements					Vibrations		
	C_2 (z)	σ_v (xz)	σ_v (yz)	Raman	I.R.	Ring	H	Total
A_1	*s*	*s*	*s*	*p*	*a*	5	6	11
A_2	*s*	*as*	*as*	*dp*	*ia*	1	2	3
B_1	*as*	*s*	*as*	*dp*	*a*	4	6	10
B_2	*as*	*as*	*s*	*dp*	*a*	2	4	6

Accordingly, many pyridinium salts have been prepared, together with their N-deuterated analogues,* and their infrared spectra in the solid state have been recorded with the high precision of a prism-grating spectrophotometer. The Raman shifts (of the HCl salt in aqueous solution), together with polarization measurements, were obtained through the courtesy of Dr. J. C. Evans, Chemical Physics Research Laboratory, the Dow Chemical Company, Midland, Michigan, U.S.A. (4). The spectra have been compared with those of C_6H_6 and C_6H_5D for which the assignments are known with precision and confidence (5–11).

EXPERIMENTAL

The Spectra

The spectra were recorded on a Perkin–Elmer model 221 spectrometer equipped with a sodium chloride prism-grating combination.

All the spectra were of the solid state as emulsions in nujol or fluorolube. Some of the salts which were hygroscopic, and all the deuterium salts, were handled in a dry box.

Preparation of the Compounds

$C_5H_5NH^+Cl^-$.—Anhydrous HCl was passed through an ethereal solution of pyridine. The resulting precipitate was recrystallized from a methanol–acetone mixture. M.p. 140–142° C; HCl: found: 31.5%, calc.: 31.6%.

$C_5H_5NH^+Br^-$.—An ether solution of pyridine was treated with anhydrous HBr to give a white solid which was recrystallized from a methanol–acetone mixture. M.p. 213–214° C; HBr: found: 51.7%, calc.: 50.5%.

$C_5H_5NH^+I^-$.—Aqueous HI was added to an ethereal solution of pyridine. The white precipitate so formed was recrystallized from methanol. M.p. 214° C (decomp.); HI: found: 63.0%, calc.: 61.8%.

$(C_5H_5NH^+)_2ZnCl_4^=$.—Acetone solutions of $C_5H_5NH^+Cl^-$ and $ZnCl_2$ were mixed and poured into ether; a white solid separated. This was identical with that formed when the pyridine:$ZnCl_2$ complex in warm acetone was treated with HCl. M.p. 179–182° C.

$(C_5H_5NH^+)_2SnBr_6^=$.—Chloroform solutions of $C_5H_5NH^+Br^-$ and $SnBr_4$ were mixed, and the resulting pale yellow precipitate was recrystallized from water. M.p. 280° C; Sn: found: 13.8%, calc.: 15.7%.

$(C_5H_5NH^+)_2SnCl_6^=$.—Chloroform solutions of $C_5H_5NH^+Cl^-$ and $SnCl_4$ were mixed, and the resulting white precipitate was recrystallized from water. M.p. 280° C; Sn: found: 26.0%, calc.: 24.0%; Cl: found: 45.0%, calc.: 43.7%.

$C_5H_5NH^+ClO_4^-$.—Perchloric acid (70%) was added slowly to liquid pyridine. The

**For the sake of brevity, the terms* H *salt and* D *salt may be employed in what follows. It is to be understood that* D *salt means* D *substitution for* H *only at the nitrogen atom.*

white precipitate so formed was recrystallized from 95% ethanol. M.p. 280–283° C; $HClO_4$: found: 56.9%, calc.: 56.0%.

$C_5H_5NH^+BF_4^-$.—Aqueous 50% HBF_4 added to pyridine gave a white solid, which was taken up in acetone and recrystallized by the addition of ether. M.p. 208–210° C.

$C_5H_5NH^+SbCl_6^-$.—Chloroform solutions of $C_5H_5NH^+Cl^-$ and $SbCl_5$, on being mixed, gave a white precipitate which was used without further treatment after washing with ether. M.p. 215–218° C; Sb: found: 29.1%, calc.: 29.5%; Cl: found: 53.0%, calc.: 51.3%.

Pyridinium reineckate.—Aqueous solutions of $C_5H_5NH^+Cl^-$ and reinecke salt were mixed. Removal of solvent gave a purple solid, which was recrystallized from ethanol. M.p. 205–209° C.

Deuterated salts.—Deuterated salts were prepared by twice exchanging with D_2O, the excess being pumped off *in vacuo* with a mercury diffusion pump.

RESULTS

The spectrum of one of the salts is shown in Fig. 1. It is actually as recorded on blank paper, after first recording on grid paper. The broken lines are drawn in by hand from the recorded spectrum of the deuterated compound, occasionally with slight alteration of background for the sake of clarity. Table II shows the bands in these spectra, while Table III shows a comparison of the assigned fundamentals with those of C_6H_6 and C_6H_5D. Bands due to polyatomic anions have been omitted since their locations are known and no new factors are involved.

DISCUSSION

It will be convenient to accept I as a model for the assignments of the cation. This has C_{2v} symmetry, requiring 30 fundamentals of which 3 would be inactive in the infrared; all of them would be Raman active. Table I shows the selection rules for the various vibrations, and Table IV describes their approximate character. The notation is that of Wilson (5), which has been followed by many authors. The use, in parentheses, of a capital *A* or *B* with subscript 1 or 2 obviously refers to the species of a vibration. When confusion would otherwise result, the frequency designation may be followed by H or D, in parentheses, to distinguish between the H salt and the D salt.

For the purpose of discussion, the frequencies of the salt $(C_5H_5NH^+)_2SnBr_6^=$ and its deuterated analogue will be used. Certain bands show trends depending on the anion. The $SnBr_6^=$ salt is approximately median with respect to these trends. It will be convenient to divide the spectra into four regions.

The 3500 to 1650 cm^{-1} Region

All the C—H and N—H stretching bands will occur in this region. The various spectra are characterized by large variations in frequency and intensity. Certain medium-intensity bands do show a consistent pattern and are to be identified as the C—H stretching modes above 3000 cm^{-1}. Of greater interest is the band which can be described as a N—H stretching vibration: this is ν_{7a}. It shows a large variation in intensity, band width, and frequency—from a very broad, intense band at 2439 cm^{-1} in $C_5H_5NH^+Cl^-$ to a sharp, medium intensity band at 3300 cm^{-1} in $C_5H_5NH^+SbCl_6^-$. In the median compound chosen, it is at 3236 cm^{-1} (with two submaxima) and in the deuterated salt it is at 2392 cm^{-1}, a ratio of 1.35. This band could then be properly designated as due mainly

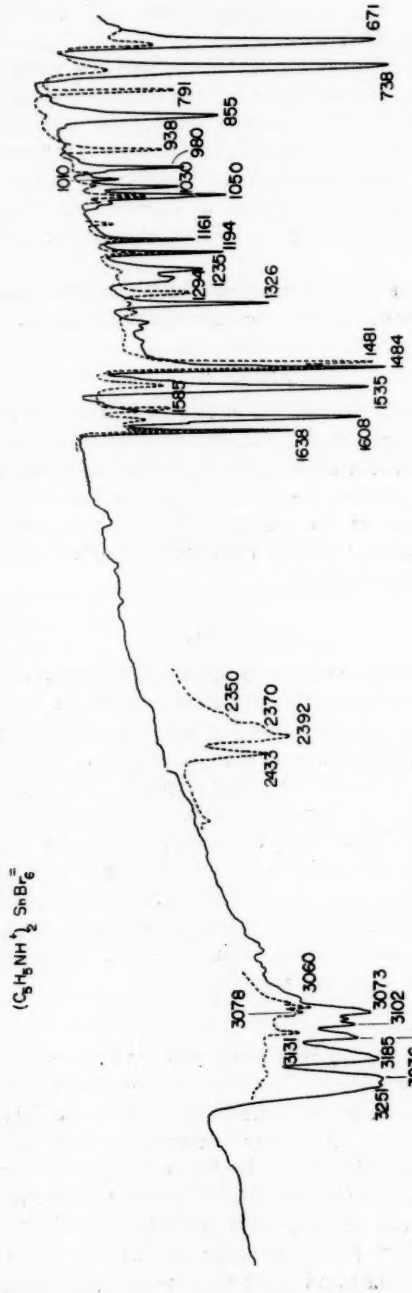


FIG. 1. Spectrum of $(C_5H_5NH^+)_2SnBr_6^=$.

to the hydrogen motion. Further discussion on the shape, intensity, and position of this band will be reserved for a later section.

The 1650 to 1400 cm^{-1} Region

All the spectra show a striking similarity in this region for all the compounds studied. Four bands of a remarkable constancy in frequency and intensity ratio are located near 1635, 1610, 1535, and 1485 cm^{-1}. This constancy in position and intensity indicates that they arise from motions unlikely to be affected by peripheral interaction, and suggests that they are due to nuclear vibrations. They have been assigned to the following vibrations respectively: ν_{8a} (A_1), ν_{8b} (B_1), ν_{19b} (B_1), ν_{19a} (A_1). The motions involved in these vibrations are visualized approximately in Fig. 2, which shows a set of normal co-ordinate

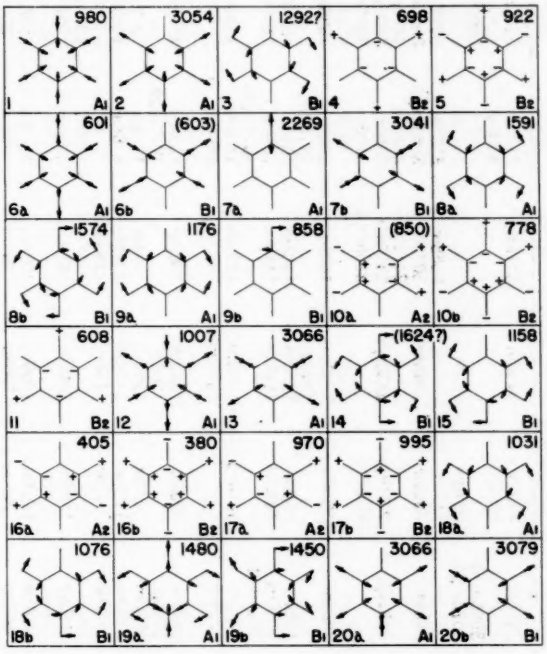


FIG. 2. Normal co-ordinate displacements for C_6H_5D.

displacements for C_6H_5D, collected from several of Ingold's papers (7). The displacements are not intended to be strictly accurate.

The Raman spectrum shows the 1635 and 1610 cm^{-1} bands to be depolarized and the 1485 cm^{-1} band probably polarized. The 1635 cm^{-1} band would be expected to be polarized completely, but even a slight polarization is consistent with a vibration of A_1 species.

The changes noticed in the N-deuterated analogue are those expected for vibrations having little or no movement of the H or D atom. The 1610 and 1535 cm^{-1} bands disappear, to be replaced by two bands at 1585 and 1475 cm^{-1}, the latter being often very close to ν_{9a} at 1485 cm^{-1}. The ratios ν_H/ν_D for ν_{8b} and ν_{19b} are 1.02 and 1.04, respectively, which are very close to those of C_6H_6 and C_6H_5D as shown in Table III.

TABLE II

Frequencies of the pyridinium ion in various salts

	Cl^-		Br^-		I^-		$ZnCl_4^=$		$SnBr_6^=$	
	H	D	H	D	H	D	H	D	H	D
	3130w	3118m	3128m	3120w	3140m	3118m	3130m	3130m	3134s	3131m
							3105m			
			3095m,sh					3090s	3088s	3088m
			3075m,sh	3070w					3073s	3078m
		3068m,sh				3065m	3070s	3070m	3060m	3061m
	3042s	3040s	3054s	3040m	3045m	3040m	3052m,sh	3055m	3045w	3052w
						2412m,b	3213vs	2420m	3251s	2433s
ν_{7a}	2439vs,b	1985vs,b	2591s,b	2120s,b	2833s,b	2300s,b	3155vs	2340vs,b	3236s	2392s
	2375vs,b	1915vs,b		2050s,b		2235m,b	3105vs	2315s	3185s	2370s
								2228m	3102s	2350m
ν_{8a}	1634m	1633m	1634m	1631m	1631s	1631s	1639s	1639m	1638s	1635m
					1610m		1616m			
ν_{8b}	1610vs	1588m	1604s	1582m	1600vs	1582m	1610s	1590m	1608vs	1585m
ν_{9b}	1532vs	1476vs	1525vs	1485vs	1525vs	1472vs	1530vs	1476vs	1535vs	1481vs
$2\ \nu_6$			1515w,sh				1513w,sh			
ν_{19a}	1488vs	1488vs	1478s	1474vs	1478vs	1478vs	1484vs	1484vs	1484vs	1486vs
$2\ \nu_{11}$	1377w		1358w		1360w		1366w			
	1342w									
ν_3	1318m	1300m	1330w	1300m	1322m	1300m	1326m	1300m	1326m	1294m
$\nu_{6a} + \nu_{6b}$			1245m		1245w		1247w,sh		1246w	
ν_{9b}	1244m	928m	1238m	927m	1235m	924m	1235m	932m	1235m	938m
ν_{9a}	1200w	1196m	1180w	1180w	1184m	1185m	1188m	1188m	1194m	1192m
ν_{15}	1162w	1160m	1150w	1150w	1148m	1150m			1161m	1160m
$\nu_{11} + \nu_{16b}$							1080w	1070w		
ν_{18b}	1060m	1058m	1052m	1055m	1050m	1050m	1050m	1050m	1050m	1050m
ν_{18a}	1030w	1030w	1029w	1025w	1029m	1025m	1028m	1025m	1030m	1025w
ν_5	1005m	812m	994m	804m	992m	793m	991m	800m	980m	791m
ν_1	995m,sh	992m	985m,sh	990m	985m,sh	989m	1007w	1008w	1010w	1008w
							910m,sh	914m,sh		
ν_{10b}	945m	720m	910w	685m	870m	685m	891m	680m	855m	680m
ν_4	750vs		750vs		741vs		750vs		738vs	
ν_{11}	680vs		678vs		671vs		675vs		671vs	

TABLE II (*Concluded*)

Frequencies of the pyridinium ion in various salts (*Concluded*)

	$SnCl_6^-$		ClO_4^-		BF_4^-		$SbCl_6^-$		$Rein^-$	
	H	D	H	D	H	D	H	D	H	D
	3140s	3139m	3148m	3140m	3148s	3148s	3151s	3140m		
	3110s				3100m	3104m	3110m	3100m		
			3090s	3096m		3090m	3095m	3090m		
	3075s	3080m		3072m			3078m,sh	3078m		
		3068m				3068w		3066w		
	3042w	3042w	3050m							
	3260s	2438s							3335s,sh	2470m,sh
ν_{7a}	3240s	2398s	3268s	2446m	3290vs	2454s	3300s	2460s	3302s	2440s
	3188s		3195s	2375s,b	3205s	2412 s	3252s	2435m		2400m
			3118m	2335m,sh	3122m	2365m	3209s	2418s		
ν_{8a}	1640s	1635m	1637m	1636m	1640s	1639m	1640m	1638m	1640m	1634m
ν_{8b}	1612vs	1586m	1610vs	1589m	1612vs	1590m	1613s	1588m	1608s	1585w
ν_{9b}	1531vs	1482vs	1538vs	1484vs	1542vs	1488vs	1541s	1492s	1538s	1478s
$2\ \nu_4$										
ν_{19a}	1482vs	1486vs	1488vs	1488vs	1490vs	1490vs	1490s	1485s	1482s	1486s
$2\ \nu_{11}$	1368w		1370w		1376w		1377w			
ν_3	1322m	1292m	1330m	1304m	1332m	1305m	1326m	1301m	1320w	1297w
$\nu_{6a} + \nu_{6b}$	1261w						1259w			
ν_{9b}	1239m	938m	1250m	930m	1255m	930m	1245w	946m	1245s	930w
ν_{9a}	1195m	1190m	1202m	1200w	1207w	1205w	1200m	1200m	1193w	1190w
ν_{15}	1164m	1159m	1170s	1170m	1174m	1172m	1168m	1164m	1161w	1160w
$\nu_{11} + \nu_{16b}$	1060w,sh						1065w	1065w	1060w	
ν_{18b}	1053m	1050m	1050vs	1050s			1050w	1050w	1048m	
	1035w									
ν_{18a}	1030m	1020w	1028vs	1028s			1030w	1025w	1029w	1025w
ν_5	982m	792m	997s	794m	990vs	797m	976m	782m	968m	780m
ν_1	1010w	1012w							1010w	1010w
ν_{10b}	862m	672m	895m,b	680m	900m,b		848m	680m	840m	680m
ν_4	740vs		748s		755s		731vs		730s	
ν_{11}	675vs		675s		681s		664vs		662s	

TABLE III

Fundamentals of benzene,* pyridinium ion, and monodeuterated* derivatives

C_6H_6					C_6H_5D			$C_5H_5NH^+$		
Raman and I.R. (gas)					Raman (liq.)	I.R. (gas)		H^+ (solid)	D^+ (solid)	
νC–H	ν_{20a}	E_{1u}	3080	I.R.	A_1	3060				
νC–H	ν_{20b}				B_1	3077	3079			
νC–H	ν_2	A_{1g}	3062	R	A_1	3055				
νC–H	ν_{13}	B_{1u}	3060	F	A_1	3060	3066	3060	3061	
νC–H	ν_{7a}	E_{2g}	3047	R	A_1	2269	2267 (1.35)	3236	2392	(1.35)
νC–D	ν_{7b}				B_1	3041		3045	3052	
νC–C	ν_{14}	B_{2u}	1309	F	B_1	1617?	1624?			
νC–C	ν_{8a}	E_{2g}	1596	R	A_1	1591		1638	1635	
νC–C	ν_{8b}				B_1	1574	(1.02)	1608	1585	(1.02)
νC–C	ν_{19a}	E_{1u}	1485	I.R.	A_1	1479	1481	1484	1486	
νC–C	ν_{19b}				B_1	1449	1452 (1.02)	1535	1481	(1.04)
δC–H	ν_3	A_{2g}	1326	F	B_1	1292		1326	1294	
δC–H	ν_{9a}	E_{2g}	1178	R	A_1	1176		1194	1192	
δN–H	ν_{9b}				B_1	857	859 (1.37)	1235	938	(1.32)
δC–H	ν_{15}	B_{2u}	1147	**F**	B_1	1158	1161	1161	1160	
δC–H	ν_{18a}	E_{1u}	1037	I.R.	A_1	1031	1030	1030	1023	
δC–H	ν_{18b}				B_1	1075	1078	1050	1050	
δC–C	ν_{12}	B_{1u}	1010	F	A_1	1007				
νC–C	ν_1	A_{1g}	992	R	A_1	980		1010	1008	
γC–H	ν_5	B_{2g}	985	F	B_2	922	922 (1.07)	980	791	(1.24)
γC–H	ν_{17a}	E_{2u}	970	F	A_2	970	*ia*	*ia*	*ia*	
γC–H	ν_{17b}				B_2	990	995			
γC–H	ν_{10a}	E_{1g}	849	R	A_2	850	*ia*	*ia*	*ia*	
γC–H	ν_{10b}				B_2	779	775 (1.09)	855	680	(1.26)
γC–C	ν_4	B_{2g}	703	F	B_2		698	738	620?	
γC–H	ν_{11}	A_{2u}	671	I.R.	B_2		608 (1.10)	671		
δC–C	ν_{6a}	E_{2g}	606	R	A_1	602				
δC–C	ν_{6b}				B_1	603				
γC–C	ν_{16a}	E_{2u}	405	F	A_2		*ia*	*ia*	*ia*	
γC–C	ν_{16b}				B_2	380	380 (1.06)			

*The data and assignments are those of Ingold and colleagues (7) except for ν_{14} and ν_{15} (8, 9, 11), which have been reassigned.

TABLE IV

Description of vibrations in different species

	A_1	A_2	B_1	B_2
C—H stretch	2, 13, 20*a*		7*b*, 20*b*	
N—H stretch	7*a*			
C—C (N) stretch	1, 8*a*, 19*a*		8*b*, 14, 19*b*	
C—H ∥ bend	9*a*, 18*a*		3, 15, 18*b*	
N—H ∥ bend			9*b*	
C—H ⊥ bend		10*a*, 17*a*		5, 11, 17*b*
N—H ⊥ bend				10*b*
C—C—C (N) ∥ bend	6*a*, 12			16*b*
C—C—C (N) ⊥ bend		16*a*		4

Although the frequency ratios between the H and D salts just mentioned seem satisfactory, the intensity changes on deuteration are not as clear. The 1610 cm^{-1} band, a strong band in the H salt, seems on occasion to be quite weak in some of the D salts. While changes like this are often real, it may be that the situation is complicated further by Fermi resonance between some overtone, or combination tone, and ν_{8b}. This would have an effect on the intensity of the band in the H salt but not in the D salt because of its new position. In the D salts some bands are seen near 1615 and 1610 cm^{-1}, which are thought to be real overtones or combination bands, not simply residual bands.

The 1400 to 1020 cm^{-1} Region

In this region there should be six in-plane hydrogen-deformation frequencies. In C_6H_6 and C_6H_5D, they are weak in the infrared. The same is true of the pyridinium ion. They have been identified and assigned as ν_3, 1320 cm^{-1}; ν_{9a}, 1194 cm^{-1}; ν_{9b}, 1235 cm^{-1}; ν_{15}, 1161 cm^{-1}; ν_{18a}, 1030 cm^{-1}; ν_{18b}, 1050 cm^{-1}. The changes that occur on deuteration are a shift of the 1320 cm^{-1} band to 1294 cm^{-1}, and a more pronounced shift of ν_{9b} from 1235 to 938 cm^{-1}. The ratio of these last two frequencies is 1.32, again identifying this vibration as predominantly an in-plane deformation of the hydrogen attached to the nitrogen atom. In C_6H_6 this band is at 1178 cm^{-1}, shifting to 859 cm^{-1} in C_6H_5D, a ratio of 1.37. The vibration ν_{9b} occurs at a very constant frequency in a large number of compounds. The corresponding band in pyrrole is at 1146 cm^{-1}, moving to 915 cm^{-1} in pyrrole-N-*d* (12). Here the ratio is 1.25, indicating the vibration to be a slightly mixed one.

The marked similarity in frequency of the in-plane hydrogen-deformation bands between C_6H_6 and $C_5H_5NH^+$ and their deuterated analogues is noteworthy. With the exception of ν_{9b}, the largest difference is 28 cm^{-1}, and the next largest is 16 cm^{-1}. This is to be expected if the proposed similarity between the two compounds exists. The largest difference between them is at the nitrogen atom. Thus the N—H motions would be expected to show the largest difference, with the other four C—H bonds being little different from C_6H_6.

There should also be in this region an in-plane carbon–carbon bending mode, ν_{12} (A_1). This is doubtless the Raman shift at 1050 cm^{-1} which is polarized and, therefore, belongs to the A_1 species. This band is unlikely to be strong in the infrared (it was not listed in the infrared spectrum of C_6H_5D vapor by Ingold); therefore, it seems preferable to retain the 1050 cm^{-1} band for ν_{18b} and regard ν_{12} as unidentified.

The 1020 to 640 cm^{-1} Region

This region will contain the ring-breathing and -bending modes as well as the characteristic out-of-plane hydrogen-deformation vibrations. The higher-frequency area in this

region is, unfortunately, not too well defined. The bands are weak, and their intensity ratios are variable in different salts. Neither is there any well-defined consistency in frequency such as that shown by the bands in the first two regions. These variations, however, though making the assignments a little more time consuming, eventually reveal trends which are important in determining intermolecular effects, which will be treated later.

A weak band in the infrared spectrum at 1010 cm^{-1} (998 cm^{-1} in the deuterium analogue) and at 1009 cm^{-1} in the Raman spectrum (the strongest band in the spectrum) can be confidently assigned to ν_1 (A_1), the totally symmetric ring-breathing mode.

Of the six out-of-plane hydrogen-bending frequencies, two belonging to the A_2 species are inactive in the infrared; these are ν_{17a} and ν_{10a}. A band at 980 cm^{-1} in $C_5H_5NH^+$, moving to 791 cm^{-1} (a ratio of 1.24) in $C_5H_5ND^+$, has been assigned to ν_5, by analogy with the 985 and 922 cm^{-1} bands in C_6H_6 and C_6H_5D. This band shows certain trends in different compounds, which will be discussed later, as does a band at 855 cm^{-1}, which has been assigned to ν_{10b}. This band disappears in the deuterated compound but a band at 680 cm^{-1} has been tentatively assigned to this vibration. Its precise identification hinges on the location of ν_4. The 738 cm^{-1} band fits this assignment reasonably well but it disappears on deuteration. The corresponding bands in C_6H_6 and C_6H_5D at 703 (forbidden) and 698 cm^{-1}, respectively, show virtually no shift on deuteration. The assignments in this region are hampered by the lack of knowledge of data below 640 cm^{-1}.

The ratios for the H and D species of ν_5 and ν_{10b} are 1.24 and 1.26 respectively, for the pyridinium ion; for C_6H_6 and C_6H_5D, they are 1.07 and 1.09. These are rather large differences but seem to be the most plausible assignments, though possible alternative ones are discussed in the next section.

This completes the assignments as far as possible at this stage. The assignments of the C—C (C—N) stretching modes and the C—H and N—H stretching modes, as well as the in-plane bending modes seem reasonably satisfactory, although the out-of-plane hydrogen-deformation vibrations and C—C (C—N) bending modes are rather more difficult to assign. One factor contributing is the absence of band shapes to assist vapor-phase assignments. This situation may be insuperable since experimental difficulties involved in recording a vapor-state spectrum seem formidable.

Alternative Assignments

Although the assignments in Table III seem satisfactory, some of the H/D ratios are a little different from the C_6H_6/C_6H_5D ratios, so that alternative assignments were considered in order to see if a more consistent set of values could be achieved. These are shown in Table V for the bands involving a shift on deuteration.

TABLE V

Alternative assignments of fundamentals

	C_6H_6	C_6H_5D	Ratio	$C_5H_5NH^+$	$C_5H_5ND^+$	Ratio
ν_{8b} (B_1)	1596	1574	1.02	1608	1585	1.02
ν_{19b} (B_1)	1485	1449	1.02	1535	1481	1.04
ν_{9b} (B_1)	1178	857	1.37	1235	(953)	(1.37)
ν_5 (B_2)				980	938	1.04
ν_{10b} (B_2)	849	775	1.09	855	791	1.08
ν_{11} (B_2)	671	608	1.10	671	(608)	(1.10)

The assignments of the 1610 and 1535 cm^{-1} bands to ν_{8b} and ν_{19b} seem unquestionably

correct. However, by displacing the other bands progressively to the next lower assignment, such that the 938 cm^{-1} band is allotted to ν_5, etc., an impressive agreement between the ratios is noticed. The figures in parentheses in Table V are calculated assuming the same ratio for the pyridinium series as for the benzene series.

The test of this set of assignments seems to be in the fate of the vibration ν_{9b} (D). If the 938 cm^{-1} band is allocated to ν_5 (D), then it is difficult to find a suitable band for ν_{9b} (D). Its calculated value would be about 953 cm^{-1}. No band near this frequency has been seen, unless it is obscured by the band at 938 cm^{-1}. (It will be recalled that ν_5 is a band which shows a trend depending on the anion.) This is unlikely since ν_5 is generally weak, and no structure can be observed in it. Moreover, it becomes even more unlikely to have to assume that ν_{9b} (D) is always hidden by ν_5 (D).

Another assumption, which begs the question, and is equally unpalatable, is that ν_{9b} (D) is so weak that it is unobservable even in thick films (or with electronic ordinate expansion). ν_{9b} for C_6H_5D is actually a medium strong band and is easily observable. It seems, therefore, that this assumption is unlikely to be valid for the pyridinium ion.

The alternative assignments quoted in Table V seem to require too many coincidences to appear plausible. They are considered simply because the other assignments seem to give H/D ratios which are not consistent between the benzene and pyridinium series. There may be good reasons why these ratios should not be so similar in the two compounds. The vibrations considered involve the nitrogen atom and the hydrogen atom attached to it. There is no doubt that this is a highly polar link, much more so than the C—H link in C_6H_6. It is, perhaps, therefore, not too unreasonable to expect different H/D ratios between the two compounds.

Comparison with Other Studies

Two previous papers have considered assignments of the pyridinium ion. Greenwood and Wade (1) made assignments after studying the hydrochloride and the tetrachloroborate; Spinner (3) published the Raman spectrum of an aqueous solution of the hydrochloride. Their results are shown in Table VI and compared with the present infrared data, and the Raman shifts measured by Evans (4).

All authors agree on the assignment of the 1634 and 1610 cm^{-1} bands to ν_{8a} and ν_{8b}. Greenwood and Wade reverse ν_{19a} and ν_{19b}, while Spinner does not record the 1535 cm^{-1} band. The presence of the almost certainly polarized band at 1490 cm^{-1}, although weak, justifies designation of this band as ν_{19a} of A_1 species rather than ν_{19b} of B_1 species. The absence of the 1532 cm^{-1} band in both Raman spectra can be taken as strong evidence that it is extraordinarily weak and, therefore, not an A_1 band.

Of the two bands at 1200 and 1244 cm^{-1}, the latter, because of its disappearance on N-deuteration, must be associated with the nitrogen—hydrogen atom. The analogy with the benzene spectrum, and the lower intensity of the Raman shift compared with the 1200 cm^{-1} band, establish the 1244 cm^{-1} band as ν_{9b} (B_1). The 1200 cm^{-1} band must then be ν_{9a} (A_1), though it is not polarized.

The Raman studies show the 1162 cm^{-1} band as depolarized and quite weak. This is in keeping with the species and assignment of ν_{15} (B_1).

The assignment of the 1030 and 1060 cm^{-1} infrared bands to ν_{18a} (A_1) and ν_{18b} (B_1) is firmly supported by the Raman shift at 1025 cm^{-1}, which is polarized and intense. The 1060 cm^{-1} band has been assigned to ν_{18b}, more by analogy with benzene than by regard to the Raman data. ν_{18b} in benzene was at 1075 cm^{-1}, but ν_{12} was not observed in the infrared vapor spectrum. It is, therefore, not inconsistent to assign the infrared 1060 cm^{-1}

TABLE VI

Fundamentals of $C_5H_5NH^+Cl^-$ by various authors

			I.R.*	I.R.†	I.R.‡	Raman‡	Raman§
ν_{N-H}	ν_{7a}	A_1	2840	2439			
			2740	2375			
ν_{C-C}	ν_{8a}	A_1	1631	1634	1630	1635 (12)‖	1636 (19) D¶
ν_{C-C}	ν_{8b}	B_1	1603	1610	1604	1615 (4)	1613 (12) D
ν_{C-C}	ν_{19a}	A_1	1530	1488	1479		1490 (2) ?
ν_{C-C}	ν_{19b}	B_1	1481	1532	1428		
δ_{C-H}	ν_3	B_1	1252	1318	1332	1334 (2)	
δ_{C-H}	ν_{9a}	A_1		1200	1189	1201 (10)	1200 (16) D
δ_{N-H}	ν_{9b}	B_1		1244	1245	1245 (3)	1254 (9) D
δ_{C-H}	ν_{15}	B_1	1198	1162	1155	1162 (2)	1163 (4) D
δ_{C-H}	ν_{18a}	A_1	1168	1030	1027	1027 (22)	1025 (50) P
δ_{C-H}	ν_{18b}	B_1		1060	1053	1060 (5)	
δ_{C-C}	ν_{12}	A_1	1059				1060 (11) P
ν_{C-C}	ν_1	A_1	1003	995	997	1010 (75)	1009 (100) P
γ_{C-H}	ν_5	B_2	940	1005	1012		
γ_{C-H}	ν_{10b}	B_2		945		952 (4)	
γ_{C-C}	ν_4	B_2	759	750	749		
γ_{C-H}	ν_{11}	B_2	684	680	682		
δ_{C-C}	ν_{6a}	A_1				639 (11)	637 (16) D
δ_{C-C}	ν_{6b}	B_1				611 (7)	611 (7) D
γ_{C-C}	ν_{16b}	B_2				393 (2)	396 (4) P

*Greenwood and Wade (1).
†This work.
‡Spinner (3).
§Evans (4).
‖Figures in parentheses refer to relative intensities.
¶Bands marked P are definitely polarized; those marked D appear to be depolarized but may be slightly polarized.

band to ν_{18b}, while assigning the polarized 1060 cm^{-1} Raman shift to ν_{12} (A_1). Greenwood and Wade have ν_{12} (A_1) at 1059 cm^{-1} and this may indeed be so.

No exception is taken to the universal assignment of the band near 995 cm^{-1} to the totally symmetric ring-breathing mode, ν_1. It is the strongest band in the Raman spectrum and is polarized although it is weak in the infrared.

The out-of-plane hydrogen deformations ν_5 and ν_{10b} are assigned chiefly on their movement upon N-deuteration. No help can be gleaned from the Raman data since no bands were observed in this region. The 952 cm^{-1} band quoted by Spinner is said by Evans to be the 1009 cm^{-1} band excited by the 4348-Å Hg line.

The intense bands at 754 and 682 cm^{-1} are unequivocally assigned to ν_4, the ring-bending mode, and ν_{11}, the out-of-plane hydrogen deformation, respectively. Although these bands are not visible in the Raman shifts, their characteristic intensity and position in the infrared spectrum are unmistakable.

The assignments of the bands having been considered, it is now convenient to examine the variations or trends in certain bands that have been briefly noted previously.

Variation of ν_{7a}

Table VII shows the variation in frequency of ν_{7a}. Some of these bands are not single bands, but have two, three, or occasionally four components. The second column in the table is the one of strongest intensity.

This band varies in frequency from 2439 cm^{-1} in $C_5H_5NH^+Cl^-$ to 3302 cm^{-1} in pyridinium reineckate. As noted earlier, it is broad and strong at low frequencies, but becomes sharper and less intense as it moves towards higher frequencies. Half widths for some of the bands at higher frequency are: $SnBr_6^=$, 68 cm^{-1}; $SnCl_6^=$, 76; ClO_4^-, 93; BF_4^-, 95; $SbCl_6^-$, 76. Undoubtedly the breadth, intensity, and low frequency are due to a strong

TABLE VII

Pyridinium ion frequencies: nitrogen—hydrogen stretching frequencies, ν_{NH}

	H	D	H	D	H	D	H	D
Cl^-			2439 (1.23)	1985	2375 (1.24)	1915		
Br^-			2591 (1.22)	2120		2050		
I^-		2412	2833 (1.23)	2300		2225		
$ZnCl_4^=$	3213 (1.33)	2420	3155 (1.35)	2340	3105 (1.34)	2315		2228
$SnBr_6^=$	3251 (1.34)	2433	3236 (1.35)	2392	3185 (1.34)	2370	3102 (1.32)	2350
$SnCl_6^=$	3260 (1.33)	2438	3240 (1.35)	2398	3188			
ClO_4^-			3268 (1.34)	2446	3195 (1.35)	2375	3118 (1.34)	2335
BF_4^-			3290 (1.34)	2454	3205 (1.33)	2412	3122 (1.33)	2365
$SbCl_6^-$			3300 (1.34)	2460	3252 (1.33)	2435	3209 (1.33)	2418
$Rein^-$	3335 (1.35)	2470	3302 (1.35)	2440	3238 (1.35)	2400		

interaction between the nitrogen hydrogen and a small anion associated with an intense force field. In the series Cl^-, Br^-, I^-, as the anion gets larger and the force field weakens as the effect of the anionic charge is diminished by more and more completed electron shells, the band is found at 2439, 2591, and 2833 cm^{-1}, respectively. For the compounds containing polyatomic anions associated with weak peripheral force fields, the frequency rises to higher values, till, with anions such as the hexachloroantimonate, it is questionable whether there is any significant interaction, and the N—H stretching frequency might be termed free.

This behavior has been noted and discussed by Chenon and Sandorfy (various amine salts) (13), Ebsworth and Sheppard (trimethylamine salts) (14), Nakanishi, Goto, and Ohashi (various amine salts) (15), and, most recently, by Mathieu and Poulet (ammonium salts) (16), who also have the most extensive list of anions. Nakanishi *et al.* were of the opinion that the tetraphenyl boron anion represented a case of a free N—H stretching frequency. Mathieu and Poulet question this, however, and quote higher frequencies than that in the $B(C_6H_5)_4^-$ salt, and consider that there may be some interaction between the nitrogen hydrogen and the π electrons of the aromatic rings. Such phenomena have been considered previously by several authors (17–19). Be this as it may, $C_5H_5NH^+B(C_6H_5)_4^-$ certainly does have a lower frequency than pyridinium reineckate, or $C_5H_5NH^+SbCl_6^-$: it absorbs at 3215 cm^{-1}.

The presence of multiple N—H stretching bands is an interesting and controversial aspect of hydrogen bonding in amine salts. It has been suggested (20) that they are due to a double minimum in the potential surface of the vibrating species, though this is unlikely to be so in the present cases. One consideration to be borne in mind is that there may be different N—H---X distances within the structure, giving rise to different frequencies. This is certainly so in the one structure that is known. In pyridinium reineckate, $C_5H_5NH^+[Cr(NCS)_4(NH_3)_2]^-$, the organic ion is surrounded by 12 sulphur atoms belonging to $N{\equiv}C{-}S^-$ groups (21). Nearest to the nitrogen—hydrogen atom, are two sulphur atoms at 3.36 Å and two at 3.60 Å. These are large distances but may be construed as very weak hydrogen bonds, giving rise to the two bands at 3238 and 3302 cm^{-1}. Preliminary data, supplied through the courtesy of Dr. C. Rérat, Bellevue, Paris, indicate a short hydrogen bond of 2.95 Å for the N—H---Cl bond in pyridine hydrochloride (22). At present it is not known whether there are other hydrogen bonds of different length in the structure.

Associated with this possibility, however, is the view that the satellites to the intense N—H stretching vibration are due to combination and difference bands between the

main fundamental and a low-frequency vibration associated with the N---X vibration in a hydrogen bond N—H---X (20).

Variation of ν_{9b}

It is rather surprising to find very little variation throughout the salts studied in the value of ν_{9b}, since it has been suggested that as a hydrogen bond is formed the stretching-vibration frequency is lowered, but the bending-vibration frequency is raised (23). The largest and smallest values are 1255 and 1230 cm^{-1}, and in the deuterated salt, 946 and 927 cm^{-1}. There is no smooth relation between ν_{9b} and ν_{7a} as the values in Tables VII and VIII show.

TABLE VIII

Pyridinium ion frequencies: in-plane and out-of-plane hydrogen-deformation frequencies

	ν_{9b}		ν_5		ν_{10b}		ν_{11}
	H	D	H	D	H	D	H
Cl^-	1244 (1.34)	928	1005 (1.24)	812	945 (1.31)	720	680
Br^-	1238 (1.33)	927	994 (1.22)	804	900 (1.31)	685	678
I^-	1235 (1.34)	924	992 (1.25)	793	870 (1.28)	685	671
$ZnCl_4^-$	1235 (1.33)	932	991 (1.24)	800	891 (1.31)	680	675
$SnBr_6^-$	1235 (1.32)	938	980 (1.24)	791	855 (1.26)	680	671
$SnCl_6^-$	1239 (1.32)	938	982 (1.24)	792	862 (1.28)	672	675
ClO_4^-	1250 (1.34)	930	997 (1.26)	794	895 (1.32)	680	675
BF_4^-	1255 (1.35)	930	990 (1.24)	797	900		681
$SbCl_6^-$	1245 (1.32)	946	976 (1.25)	782	848 (1.25)	680	664
$Rein^-$	1245 (1.34)	930	968 (1.24)	780	840 (1.24)	680	662

A study of the hydrogen-bending mode in trimethylamine-N-oxide salts has revealed (24) a difference of 15 cm^{-1} between the hydrochloride (1525 cm^{-1}) and the hydrobromide (1510 cm^{-1}), though four other salts ($B(C_6H_5)_4^-$, BF_4^-, ClO_4^-, and I^-) were reasonably constant around ~1500 cm^{-1}. There is a difference of ~700 cm^{-1} in the stretching vibration. Again it should be noted that the hydrofluoride has not been studied yet, and there may be a large difference between this and other salts. The situation is very similar in the trimethylamine salts as Table IX shows. The ammonium salts show

TABLE IX

Hydrogen-bending frequencies in some amine salts

	Me_3NOH^+	Me_3NH^+	NH_4^+	$C_5H_5NH^+$
F^-			1485	
Cl^-	1525	1482	1393	1245
Br^-	1510	1476	1385	1230
I^-	1498	1474	1388	1235
ClO_4^-	1505			1250
BF_4^-	1507			1255
BPh_4^-	1490	1478		

quite clearly a large difference between the fluoride and the other halides, which has been discussed by Plumb and Hornig (25).

Thus is would seem that while the hydrofluorides and hydrochlorides of these bases may have higher bending frequencies, salts with other anions show no systematic variations but minor differences due to coupling or mechanical interaction. Not enough types of

salts have been studied to see whether this behavior is the exception rather than the rule. Mathieu and Poulet's paper unfortunately gave no data on the bending modes. Miller and Wilkins (26), however, showed that for about 15 ammonium salts there was only a small variation of about ± 15 cm^{-1} in the bending frequency.

Variation of ν_5, ν_{10b}, and ν_{11}

ν_5, ν_{10b}, and ν_{11} are out-of-plane hydrogen-deformation vibrations (see Table VIII). They all show variations with the nature of the anion, that of ν_5 being the most convincing. A smooth relation between ν_5 and ν_{7a} suggests a connection between them. This is in line with the expectation that deformation frequencies get smaller, as stretching frequencies get larger, as hydrogen bonds get weaker. The total variation in ν_5 is really quite small, about 35 cm^{-1}, though most of this occurs in the last two salts, the hexachloroantimonate and the reineckate.

The case of ν_{10b} is a little more difficult. A total variation of about 105 cm^{-1} is observed, but much of the variation is random, without the fairly smooth relation that ν_5 exhibits. ν_{11} shows quite a small variation, only 20 cm^{-1}, again mostly to be seen in the last two compounds in the list, the hexachloroantimonate and the reineckate. Neglecting these two, the variation is 11 cm^{-1}, which cannot be called significant.

These variations in the bands just considered, at first sight seem to support the view that deformation frequencies rise as hydrogen bonds get stronger. However, because of the large deviations from a linear relationship between the stretching frequency, ν_{7a}, and the deformation frequency, this view must be taken as tentative at this stage. The near constancy of ν_{9b} and ν_{11} also does not support this view. There may be mechanical factors involved, too, which could complicate the matter.

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SOME FACTORS AFFECTING THE KÖNIGS–KNORR SYNTHESIS OF GLYCOSIDES[1]

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ABSTRACT

Glycosyl halides decompose readily in the presence of silver oxide under conditions generally used for the Königs–Knorr synthesis of glycosides. This "side reaction" probably accounts for the low yields commonly obtained in the synthesis of oligosaccharides, particularly those involving the formation of a glycosidic union through an unreactive secondary position. The rate of the side reaction, as well as that of the normal condensation reaction, is dependent on the condition of the silver oxide, and is retarded markedly by elemental iodine. This latter effect appears to be related to the improved yield obtained in Königs–Knorr syntheses when iodine is used. Among the products formed in the decomposition of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide are 2,3,4,6-tetra-*O*-acetyl-D-mannose, a heptaacetyl 1,2,1′-linked dimeric orthoacetate, and a second high-molecular-weight compound that also shows ortho ester properties.

The Königs–Knorr reaction (1)—the condensation of a glycosyl halide with a hydroxylic compound in the presence of a catalyst or acid acceptor—constitutes a method of high stereochemical selectivity for the synthesis of glycosides. Several detailed reviews and articles deal with the mechanism of this reaction and its many applications (2–9). Although glycosides of simple alcohols and phenols may usually be prepared in high yield, the method is less generally effective for oligosaccharides. This is true particularly when the hydroxylic component is relatively unreactive. Yields of disaccharides thus are optimal (60–75%) when the glycosyl unit is coupled with a primary hydroxyl group (5, 10, 11), and much lower (frequently less than 10%) when the condensation involves an anomeric or a secondary hydroxyl group (5, 12, 13).

Generally, the synthesis of oligosaccharides involves the use of an "inert" solvent (e.g., chloroform or benzene), a desiccant to minimize hydrolysis of the glycosyl halide, and a condensing agent. Most commonly, the condensing agent is silver oxide or carbonate. Using these normal Königs–Knorr conditions for preparing certain disaccharides we have found that a reaction occurs readily between the acetohalogen sugar and the condensing agent *even in the absence of an added hydroxylic component.* Thus, addition of silver oxide to a solution of tetra-*O*-acetyl- (or *O*-benzoyl-) α-D-mannopyranosyl or D-glucopyranosyl bromide in dry benzene or chloroform resulted in a rapid drop in the optical rotation of the solution (Table I). Chromatographic examination of the reaction mixture at intervals (after prior treatment with sodium methoxide) showed that this drop was caused by decomposition of the halide and that derivatives of the parent hexose (i.e., D-mannose or D-glucose) were being formed.* The amount of rotatory change, to a maximum value, depended on the proportion of silver oxide used (Table I), and when this maximum change had occurred, little, or none, of the glycosyl halide remained intact. However, there was a wide variation in the activity of oxide samples from different

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**The glycosyl halides themselves are converted by excess sodium methoxide to the methyl glycosides. Hence, both the parent sugar and glycoside were detected in varying proportions at intermediate stages of the reaction between the halides and silver oxide (see footnotes, Table I).*

TABLE I

Rotatory changes on treatment of tetra-*O*-acetyl-α-D-glycosyl bromides with silver oxide

Halide	Solvent	Ag_2O/halide, equivalents	Observed rotation* in deg (and time in hr)		
D-Mannose	Benzene	1.4	5.50 (0)†	4.78 (1.5)	4.69 (18)
		4.0	"	2.38 (0.8)	2.27 (18)†
		8.0	"	0.43 (0.5)	−0.03 (18)§
		16.0	"	—	−0.17 (18)
		4.0‖	3.98 (0)	0.42 (7)	0.10 (18)
D-Mannose	Benzene–pyridine (75:25)	—	4.48 (0)	4.40 (6)	3.94 (18)
D-Mannose	Chloroform	4.0	3.33 (0)	1.56 (2.5)	−0.05 (10)
		8.0	"	0.64 (2.5)	−0.04 (10)
		4.0‖	3.28 (0)	0.94 (1)	0.06 (12)
D-Mannose	Tetrahydrofuran	4.0	3.14 (0)	2.18 (0.5)	0.28 (3)
		8.0	"	0.74 (1.0)	−0.08 (3)
		4.0‖	"	1.14 (1.0)	−0.40 (10)
D-Mannose (benzoate)	Benzene	8.0	1.56 (0)	−3.80 (5)	
D-Glucose	Benzene	8.0	5.70 (0)†	1.20 (1)§	
		8.0‖	"	1.95 (1)	

*α_D (1 dm); the concentration was generally 5–10%.
†Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed methyl glycoside, no hexose.
‡Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed methyl glycoside *plus* hexose.
§Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed hexose, no methyl glycoside.
‖Silver carbonate.

sources, as shown by the varied amount of rotatory change produced* (Table II). The glycosyl halides were vigorously decomposed also with silver carbonate as well as in other solvents (e.g., tetrahydrofuran), but were much more stable in the presence of a soluble base, pyridine. These effects suggested that the metallic oxides serve as catalysts of the observed "side reaction", possibly as in the solvolysis of alkyl halides (16, 17).

TABLE II

Variation in activity of silver oxide (tetra-*O*-acetyl-α-D-mannosyl bromide (50 mg) in benzene (2 ml) containing Drierite (0.7 g) and silver oxide (25 mg); initial α_D 1.95° (0.5 dm))

Silver oxide	α_D° (5 hours)*
Prepared according to ref. 14	−0.26†
Prepared according to ref. 28	0.65
Commercial sample 1	0.69
Commercial sample 2	0.90
Commercial sample 3	1.43‡

*At 18 hours the rotational values were virtually unchanged.
†Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed mannose, no methyl mannoside.
‡Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed mannose *plus* methyl mannoside.

The reaction products isolated after treatment of the acetylated glycosyl halides with silver oxide in benzene were amorphous solids essentially free from halogen. They did

**Helferich and Klein (14) have noted that the effectiveness of silver oxide in disaccharide syntheses varies with the method of preparation of the oxide. The age of the oxide may also be important, and hence the literature contains frequent references to the use of a "freshly-prepared" sample. Two of the samples tested in the current study were found to be stable over a period of 1 year whereas the activity of a third sample decreased slowly. The importance of using properly prepared silver chloride for converting an α-D-glycosyl bromide to the β-D-chloride (15) is perhaps a related example of how the condition of a silver salt may affect the reactions of glycosyl halides.*

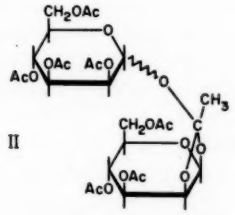
not exhibit unsaturated properties and absorbed weakly in the infrared region characteristic of hydroxyl groups. Elemental analysis data suggested that formation of these products involved the introduction of one oxygen atom per two tetraacetyl hexosyl residues (R), as represented by the reaction

$$2RBr + Ag_2O \rightarrow ROR + 2AgBr.$$

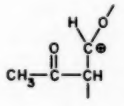
Consistent with this possibility was the finding that the reaction products contained a high proportion of dimeric (and possibly trimeric) components. These components were readily detected chromatographically, after deacetylation with sodium or barium methoxide, as material travelling at the rate of disaccharides and higher oligosaccharides. However, the free hexose was the major product found on the chromatograms.

By column chromatography on silicic acid, several fractions were obtained from each reaction product. Those fractions derived from the D-glucosyl bromide were syrups, but in the D-mannose series three crystalline compounds were isolated. Of these, the major identifiable product was 2,3,4,6-tetra-*O*-acetyl-D-mannose (I) (isolated as the α-anomer), accounting for about 25% of the total. The two other products (II and V) each amounted to about 10% of the mixture and, on deacetylation, were found to correspond chromatographically to two of the slow-moving materials referred to in the preceding paragraph.

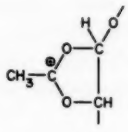
The total acetyl content (C-methyl groups) of compound II was greater than the *O*-acetyl content, the ratio of the two values being 8 to 7. When deacetylated with sodium methoxide, II afforded a crystalline material (III) which was reconverted to II by acetylation. Compound III was easily hydrolyzed by cold dilute acid to mannose and a syrupy product (IV) which, chromatographically and from a comparison of infrared absorption spectra, was indistinguishable from 2-*O*-acetyl-D-mannose (18, 19). These properties, together with the C-methyl content of III, suggested that III is composed of two D-mannose units linked by an orthoacetate structure, and that II is the corresponding heptaacetate. Further evidence for the presence of an ortho ester grouping in II was the rapid rotatory change produced by treatment with dilute methanolic hydrogen chloride, which closely paralleled that observed for other *O*-acetyl orthoacetates of D-mannose (19, 20, 21). As with tri-*O*-acetyl-D-mannose methyl 1,2-orthoacetate, the methanolysis yielded a product which appears to be 3,4,6-tri-*O*-acetyl-D-mannose (20, 22). Methylation of III via the liquid-ammonia procedure (23) afforded a crystalline ether which on hydrolysis yielded a tri- and a tetra-methyl derivative. From these data the structure of II is tentatively assigned a 1,2,1′-orthoacetyl linkage, as in formula II (Ac = CH_3CO).



II



VII



VIII

The molecular weight of V suggested that this product also is dimeric, and with methanolic hydrogen chloride the compound showed properties closely similar to those observed with II. On deacetylation with sodium methoxide, V yielded the crystalline

compound VI, which on a paper chromatogram was much slower than III and contained slightly less than one C-methyl group per two hexose units. Gentle acid hydrolysis of VI afforded mannose and a syrupy reducing ester which, however, was not 2-*O*-acetyl-D-mannose. This ester, on deacetylation with sodium methoxide, yielded non-reducing components as well as mannose. Hence V is not an isomer of II, although it also shows ortho ester properties. A more complete characterization of these new ortho esters and their various degradation products is being carried out. For the present purposes, however, it is clear that at least two types of high-molecular-weight ortho esters are produced during the decomposition of tetra-*O*-acetyl-α-D-mannosyl bromide in the presence of silver oxide.

The high-molecular-weight products formed in the decomposition of the D-glucosyl bromide also appear to be ortho esters since, as noted earlier, they are stable to methoxide ion, but were found to be hydrolyzed easily by acid. It is presumed, therefore, that the compounds are structurally akin to the D-mannose derivatives II and V.

Water appears to play a role in the formation of these products. Thus, tetra-*O*-acetyl-D-mannose may be derived from the bromide by hydrolysis. In the presence of silver ion this would be expected to involve dissociation of the bromide to yield a carbonium ion (e.g., partial structures VII and/or VIII) (6, 7, 8). Reaction, in turn, of the tetraacetate with VIII could yield an orthoacetate, such as II, but the formation of V appears to involve additional pathways. The practical difficulty of achieving completely anhydrous conditions (24),* together with the high efficiency of water in solvating carbonium ions, makes it possible that the water required for such reactions could be derived from sources such as the solvent, silver oxide,† or atmosphere. Since much of the reaction product is unaccounted for, there is the possibility also that water is produced by reaction of the silver oxide with hydrogen bromide, the elements of which come from the glycosyl halide. Alternatively, in view of the catalytic aspects of the reaction, it is not inconceivable that compounds such as II and V are produced on the surface of the silver oxide without the involvement of D-mannose tetraacetate, and that the latter is formed, at least partially, by hydrolysis of labile intermediates during processing of the reaction products. In any event, all attempts made in the present study to exclude and remove moisture from the reaction mixtures, including the precautions generally employed in Königs–Knorr syntheses (5), were ineffective in preventing the side reactions.

That side reactions of the same type occur during Königs–Knorr syntheses was shown by condensing the aforementioned glycosyl halides with 1,2,3,4-tetra-*O*-acetyl-β-D-glucose under conditions similar to those recommended by Reynolds and Evans (11). When the reaction mixtures were examined chromatographically at intervals, after prior treatment with sodium methoxide, it was found that a high proportion of the acetohalogen sugar present initially could not be accounted for either as synthesized gentiobiose or as unchanged material (i.e., as the methyl glycoside). With the D-mannosyl bromides the effect was more clearly evident from the appearance of an increasing proportion of free mannose.

Since water is a by-product of these syntheses, i.e.,

$$2RX + 2R'OH + Ag_2O \rightarrow 2ROR' + 2AgX + H_2O,$$

side reactions may be expected to occur even more readily when the hydroxylic component

*It is possible that the reaction of these glycosyl halides with silver oxide is related to the rotational changes exhibited by solutions of glycosyl chlorides of the "unstable" series, i.e., by 1,2-trans-(e,e)-chloride-acetates (see ref. 6). Recent evidence (24) shows that these changes are due not to anomerization but to hydrolysis by traces of moisture present in nominally "dry" solvents. Hydrolysis of the more stable halides used in the current study may occur in the same media only when catalytic agents, such as silver oxide, are present.

†Silver oxide is reported to adsorb hydroxyl ions strongly during preparation (25).

is present than when it is absent. To keep the concentration of liberated water to a minimum, Reynolds and Evans' procedure calls for slow addition of the glycosyl halide to the alcohol component in the presence of the condensing agent and a highly efficient desiccant. In this way the alcohol component is present in large excess (particularly at the outset) as for the preparation of simpler glycosides. Isbell and Frush (3) showed that when the method of addition is reversed, the yield of the normal condensation product is lowered, and suggested that interference by water then becomes marked. Thus, when methanol was added slowly to a solution of acetobromo-α-D-mannose or -glucose in the presence of silver carbonate (but not a desiccant) a high yield of the respective hexose tetraacetate was obtained (3). However, since the glycosyl halide and condensing agent were brought together in the absence of a large proportion of the hydroxylic component, the observed effects were probably caused, partly at least, by side reactions such as described above. *Hence, the practice of adding the glycosyl halide slowly to excess of the alcohol appears to be advantageous for minimizing side reactions having different origins.* The situation should be more difficult when the hydroxylic component is unreactive. Side reactions then may occur preferentially irrespective of whether or not water has been formed through glycoside synthesis, leaving little glycosyl halide for the desired condensation. This effect probably accounts for the poor yields of disaccharides obtained, as noted earlier, particularly in attempts to form a glycosidic union through a hindered secondary position.*

A preparation of silver oxide that promotes rapid decomposition of a glycosyl halide is also a vigorous Königs–Knorr condensing agent. Thus, by using one silver oxide sample (commercial sample 1, Table II) for the above synthesis of gentiobiose a reaction period of 2 hours was sufficient, as shown by the fact that only a trace of the D-glucosyl halide remained after that time, whereas the reaction period had to be extended to 70 hours for a less active silver oxide (sample 3). Therefore, the optimum duration of a Königs–Knorr reaction, or the optimum proportion of oxide to be used, may vary greatly, and experimental details of various recorded syntheses should not be regarded as universally applicable. According to the present data (Table II), however, the effectiveness of a given sample of silver oxide may be appraised in advance by polarimetric measurement of its reaction with the glycosyl halide to be used.

The use, as described by Helferich, Bohm, and Winkler (27), of elemental iodine as a catalyst in Königs–Knorr reaction mixtures is often followed, and makes for substantially increased yields (5, 11, 12, 21, 27). Since glycosyl halides may well react in these syntheses by an S_N1 mechanism (7, 8, 19), it has been suggested (7) that the effect of iodine is to increase the polarity of the solvent and hence the reaction rate. Although iodine is highly beneficial in the synthesis of gentiobiose (11), it was found during the experiments reported above that the disaccharide actually is formed more *slowly* in the presence of iodine than in its absence. However, iodine also suppresses the side reaction between glycosyl halides and silver oxide (Table III), a highly active sample of the oxide decolorizing the iodine solution more rapidly than a less active sample. Possibly, the side reaction is hindered more effectively in this way than is the condensation step, thereby leading to an enhanced yield of the desired product. When the proportion of iodine is low, the side reaction should be prominent (Table III), but there may be insufficient silver oxide for complete condensation if the ratio is too high. It follows that there should be an optimum proportion of iodine for Königs–Knorr syntheses, which may

**As shown by Curtis and Jones (26), it may be advantageous to use as the hydroxylic components, acyclic derivatives, the secondary hydroxyl groups of which are usually more reactive than those of related cyclic derivatives.*

TABLE III

Effect of iodine on the decomposition of tetra-*O*-acetyl-α-D-glycosyl halides in the presence of silver oxide*

Halide	Iodine	α_D in deg (and time in hr)		
D-Mannose	—	1.93 (0)†	0.18 (1)	−0.09 (18)‡
	+(25 mg)	"	0.90 (1)	0.10 (18)
	+(50 mg)	"	1.40 (1)	0.71 (18)§
D-Glucose	—	2.70 (0)†	0.70 (4)	0.69 (18)‡
	+(50 mg)	"	1.91 (4)	1.04 (18)§

*Glycosyl halide (50 mg) in benzene (2 ml) containing silver oxide (100 mg).
†Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed methyl glycoside, no hexose.
‡Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed hexose, no methyl glycoside.
§Paper chromatographic examination of the deacetylated (with sodium methoxide) material showed methyl glycoside *plus* hexose.

not coincide with the proportion generally used on the assumption that the iodine functions as a catalyst. Further, if iodine does in fact competitively inhibit the side reaction, there remains the possibility that more efficient inhibitors will be found.

EXPERIMENTAL

Solvents were purified and dried by standard methods. Silver oxide was prepared according to Young (28) and Helferich and Klein (14), or obtained commercially, and was dried in a high vacuum at 80° C. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide was prepared by the procedure of Talley, Reynolds, and Evans (21), and the corresponding benzoyl derivative according to Ness, Fletcher, and Hudson (29).

Paper chromatography was carried out with Whatman No. 1 paper using (A) butanol–ethanol–water (40:11:19) and (B) ethyl acetate – pyridine – water (10:4:3) as solvents, and *p*-anisidine hydrochloride (30) or ammoniacal silver nitrate (31) as spray reagents.

Silicic acid used for column chromatography was washed with water, then acetone, and dried at 110° C.

O-Acetyl was determined by a semimicro adaptation of the procedure of Kunz (32).

Optical rotations were measured at 25°±2°. Melting points are corrected.

Evaporations were carried out *in vacuo* at 40° C.

Treatment of 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl Bromide with Silver Oxide

A suspension of silver oxide (4.0 g) and D.icrite (6.0 g) in benzene (15 ml) was shaken for 2 hours, the D-mannosyl bromide (1.5 g) was added, and the mixture was shaken for 18 hours; α_D −0.97° (0.5 dm). The solids were filtered off and washed well with benzene and the filtrate was concentrated. An amorphous solid was obtained and dried *in vacuo*, first at 80° for 2 hours and then at room temperature for 18 hours. Yield, 1.0 g. Calculated for $(C_{14}H_{19}O_9)_2O$: C, 49.56%; H, 5.65%. Found: C, 49.72%; H, 5.66%; bromine, 0.30%.

Chromatographic examination of the material after deacetylation with sodium methoxide showed (*p*-anisidine spray) the presence of mannose, two lesser components at $R_{mannose}$ 0.72 and 0.42, and a trace component at $R_{mannose}$ 0.79. No other products were detected with the silver nitrate spray.

Ortho Ester Acetate II

The amorphous product (19.7 g) obtained by treating the D-mannosyl bromide (24.0 g) with silver oxide in benzene,* as above, was dissolved in chloroform (25 ml) and trans-

Compound II was also obtained (6% yield) when chloroform was used as the solvent.

ferred to a silicic acid column (6×50 cm), and the column was washed with chloroform. The first major fraction eluted (1.5 g) crystallized from alcohol and after recrystallization had m.p. 152–154°; $[\alpha]_D$ −23.6° (*c*, 1.2, $CHCl_3$). Calculated for $C_{28}H_{38}O_{19}$: C, 49.56%; H, 5.65%; C-methyl (8 groups), 31.7%; *O*-acetyl (7 groups), 44.4%. Found: C, 49.53%; H, 5.78%; C-methyl, 30.52%; *O*-acetyl, 44.78%.

Methanolysis of II

The ortho ester acetate (16.7 mg) was dissolved in chloroform–methanol (0.8 ml, 1:1); α_D −0.16° (1 dm). Methanolic hydrogen chloride (0.1 ml, 1.5%) was added and the rotation of the solution was then α_D 0.28° (3 min), 0.33° (5 min), 0.34° (15 min). Excess of ethereal diazomethane was added and the solution was found chromatographically (solvent A) to contain a major component corresponding to 3,4,6-tri-*O*-acetyl-D-mannose (20, 22). Evaporation of the solution and treatment of the residual syrup with excess lead tetraacetate in acetic acid showed this component to be oxidized readily to yield a product which travelled more rapidly on a paper chromatogram and gave a typical pentose color with the *p*-anisidine spray, showing that the cleavage had occurred between carbons-1 and -2.

Ortho Ester III

Compound II (370 mg) was suspended in methanol (3 ml) and treated under reflux for 10 minutes with a slight excess of sodium methoxide. During this period the compound dissolved, and when the solution was cooled, a precipitate (170 mg) was obtained, which was crystallized from water–methanol; m.p. 120°; $R_{mannose}$ 0.72. Calculated for $C_{14}H_{24}O_{12}.CH_3OH$: C, 43.27%; H, 6.78%; C-methyl (1 group), 6.5%; methoxyl, 7.5%. Found: C, 43.43%; H, 6.73%; C-methyl, 7.34%; methoxyl, 8.23%.

Ortho ester III (138 mg) in pyridine (5 ml) was treated with acetic anhydride (3 ml) for 5 hours, the reaction mixture was decomposed with ice water, and the solid product was collected. Yield, 159 mg. After recrystallization from ethanol, the product had m.p. 152–154° C, $[\alpha]_D$ −25.9° (*c*, 1.1, $CHCl_3$).

Hydrolysis of III

The ortho ester (III) (200 mg) was dissolved in 0.1 *N* sulphuric acid (4 ml), and after 2 hours the solution was neutralized with Dowex-1 resin (bicarbonate form) and concentrated. The residual syrup was found to contain approximately equal proportions of mannose and a second component which travelled at the rate of 2-*O*-acetyl-D-mannose. By chromatography on sheets of Whatman 3 MM paper, using solvent A, the mixture was separated, and the unknown syrupy component was found by infrared spectroscopy (potassium bromide disk) to be indistinguishable from the known syrupy D-mannose monoacetate. The latter was prepared from 3,4,6-tri-*O*-acetyl-D-mannose-1,2-orthoacetate by the procedure used for conversion of the corresponding L-rhamnose ortho ester to 2-*O*-acetyl-L-rhamnose (18).

Methylation of III

The deacetylated ortho ester (III) (342 mg) in liquid ammonia (15 ml) was treated with a slight excess of potassium (23), the ammonia was evaporated off, and the residue was heated under reflux in methyl iodide (5 ml) for 3 hours. After evaporation of the methyl iodide, the methylation procedure was repeated twice. The final product (348 mg) was crystallized from *n*-hexane; m.p. 142–144° C, $[\alpha]_D$ −36.5° (*c*, 1.0, $CHCl_3$). Calculated for $C_{21}H_{38}O_{12}$: C, 52.27%; H, 7.94%; methoxyl, 45.0%. Found: C, 52.21%; H, 7.93%; methoxyl, 44.1%.

The methylation product (4.8 mg) in methanol (0.5 ml) (α_D −0.10°, 0.5 dm) was treated with methanolic hydrogen chloride (0.05 ml, 6%), which caused a change in rotation to α_D 0.35° (2 min), 0.40° (5 min). After neutralization with ethereal diazomethane, the solution was found by paper chromatography to contain two components corresponding to 3,4,6-tri- and 2,3,4,6-tetra-*O*-methyl-D-mannose, respectively. Assignment of a 3,4,6-structure to the former was supported by the fact that this component was readily oxidized by lead tetraacetate in acetic acid, yielding a faster-moving product (presumably trimethyl arabinose); the tetramethyl derivative was unaffected by this treatment. When the methylation product was treated under reflux with 1% methanolic hydrogen chloride, the two products formed corresponded, as indicated by gas–liquid chromatography, to a tri- and a tetra-*O*-methyl methyl glycoside, respectively.

2,3,4,6-Tetra-O-acetyl-α-D-mannose (I)

By continued washing of the silicic acid column (above) with chloroform, a second major fraction was obtained (5.4 g), which was crystallized from a highly concentrated solution in ether; m.p. 91–92° C, $[\alpha]_D$ 25.2° (*c*, 1.4, $CHCl_3$) (lit. (33) m.p. 93°, $[\alpha]_D$ 26.3°). Calculated for $C_{14}H_{20}O_{10}$: C, 48.27%; H, 4.79%; acetyl, 49.5%. Found: C, 48.53%; H, 5.83%; acetyl, 49.23%.

On acetylation with acetic anhydride – sodium acetate the compound afforded 1,2,3,4,6-penta-*O*-acetyl-β-D-mannose; m.p. 116–118°, $[\alpha]_D$ −24.5° (*c*, 1.2, $CHCl_3$).

Ortho Ester Acetate V

When the silicic acid column was washed with chloroform–ether (3:1) a fraction (2.1 g) was eluted, which crystallized, and was recrystallized from ethanol; m.p. 216–219°, $[\alpha]_D$ −26.5° (*c*, 1.1, $CHCl_3$). Found: C, 50.00%; H, 5.66%; acetyl, 42.77%; molecular weight (Rast), 753.

In another experiment, this ortho ester also was isolated directly from the reaction mixture. Silver oxide (20 g) in benzene (100 ml) was heated under reflux for 1 hour and then approximately 50 ml of the benzene was distilled off. Drierite (30 g) was added to the cooled suspension, followed by acetobromo-α-D-mannose (7.5 g) in benzene (50 ml). After being shaken for 10 hours, the reaction mixture (α_D −0.30°) was filtered, and the solution concentrated, affording an amorphous solid (5.0 g). The latter, on being dissolved in warm ethanol, yielded crystalline material (0.58 g), m.p. 206–208° C. Recrystallization from ethanol gave a melting point of 208–211°, undepressed by admixture with the ortho ester described in the preceding paragraph. Found: C, 50.11%; H, 5.69%; C-methyl, 27.51%; molecular weight (Rast), 705; (isothermal distillation (34), acetone), 690.

The ortho ester (22.6 mg) was dissolved in chloroform–methanol (1.0 ml, 1:1); α_D −0.51° (1 dm). On addition of methanolic hydrogen chloride (0.5 ml, 1%) to the solution, the observed rotation was α_D 0.20 (2 min), 0.38 (5 min), 0.43 (25 min). Neutralization of the solution with silver carbonate, followed by paper chromatographic examination, showed the presence of a major component corresponding to 3,4,6-tri-*O*-acetyl-D-mannose, this component being readily oxidized by lead tetraacetate in acetic acid.

Ortho Ester VI

Ortho ester acetate V (500 mg) was suspended in methanol (8 ml) and treated under reflux for 10 minutes with a slight excess of sodium methoxide. The clear solution, on being cooled, deposited a precipitate which was crystallized from water–ethanol; m.p.

156–157° C, $R_{mannose}$ 0.41, $[\alpha]_D$ −5.4° (*c*, 1.1, H_2O). Found: C, 45.15%; H, 6.42%; C-methyl, 5.85%.*

The deacetylation product (V) (194 mg) was treated with 0.1 *N* sulphuric acid (4 ml) for 3 hours at room temperature, and the solution was neutralized with Dowex-1 (bicarbonate form) and evaporated. Paper chromatographic examination of the product showed the presence of mannose and an equally prominent second component, $R_{mannose}$ 0.7 (solvent A) or $R_{mannose}$ 1.2 (solvent B). This mixture was separated on Whatman 3 MM paper affording the unknown component as a syrup (86 mg). The latter absorbed strongly in the infrared regions characteristic of acyl groups and, on treatment with excess sodium methoxide, yielded mannose and two non-reducing products having $R_{mannose}$ 2.5 and 2.8, respectively (solvent A).

Treatment of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide with Silver Oxide

To a solution of the bromide (0.6 g) in benzene (7 ml) was added Drierite (3.0 g) and silver oxide (2.0 g), and the mixture was shaken for 16 hours; during this period the observed rotation changed from α_D 20.2° to 1.75° (1 dm). The solids were filtered off and washed with benzene, the filtrate was evaporated, and the amorphous solid obtained was dried in a high vacuum. Yield, 0.4 g. Calculated for $(C_{14}H_{19}O_9)_2O$: C, 49.56%; H, 5.65%. Found: C, 50.15%; H, 5.67%; bromine, 0.28%. On deacetylation with barium methoxide the product yielded glucose and two lesser-reducing components (*p*-anisidine spray) having $R_{glucose}$ 0.7 and 0.1, respectively. The latter two were hydrolyzed when the deacetylation mixture, acidified with acetic acid, was heated briefly.

The product was chromatographed on a column of silicic acid, using chloroform and chloroform–ether (varying proportions) as the eluting solvents. Several syrupy fractions were obtained, none of which, however, afforded crystalline material.

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**In another experiment the product obtained was highly hygroscopic; m.p. 180°. Found: C, 43.68%; H, 6.42%; C-methyl, 6.45%.*

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ALIPHATIC CHEMISTRY OF FLUORENE

PART VII. SPIROLACTONES[1]

P. M. G. BAVIN[2]

ABSTRACT

A number of spirolactones have been prepared from fluorene-9-carboxylic acid, either by the halolactonization reaction or by alkylation of the methyl ester with hydroxyalkyl halides.

The ease with which methyl fluorene-9-carboxylate anion (I*a*) reacts with a wide range of alkyl halides (1, 2, 3) prompted attempts to prepare 9-alkyl derivatives of the ester-hydrochloride (II), which has found use as an anti-spasmolytic agent (4) under the name Pavatrine (5, pp. 259–261). Attempts to alkylate the anion I*b* or to effect trans esterification of methyl 9-alkylfluorene-9-carboxylates with sodium 2-diethylaminoethoxide gave only traces of the desired products, but led indirectly to the preparation of some spirolactones.

I*a*, R = CH_3
I*b*, R = $CH_2CH_2N(C_2H_5)_2$

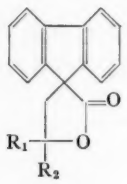
II

The reaction between 2,3-dihydroxypropyl chloride and either of the anions I*a* or *b* gave the hydroxy-γ-lactone III*b* in good yield. The unsubstituted γ (III*a*) and δ (IV*a*) lactones were prepared similarly, by alkylating I*a* with 2-bromoethanol and 3-chloropropanol,* respectively. The phenyl γ-lactone (III*c*) was obtained by reducing methyl 9-ω-phenacylfluorene-9-carboxylate (6) with sodium borohydride. The carbonyl stretching frequencies of chloroform solutions of these lactones and the related compound V*a* (7), isomeric with III*a*, agree well with the generally accepted values (8, pp. 159–160).

The hydroxylactone (III*b*) was first reported (3) as having the isomeric δ-structure (IV*b*), on the basis of the carbonyl stretching frequency of a sample prepared as a pressed potassium bromide disk. However, the infrared spectrum of a chloroform solution indicated the γ-lactone structure (III*b*), and this has been confirmed by reaction with thionyl chloride to give the chloro-γ-lactone (III*d*) (cf. reactions of the hydroxylactones derived from stilbene-2-carboxylic acids (9)). The chlorolactone has also been prepared from 9-allylfluorene-9-carboxylic acid (below). Structure III*b* for the hydroxylactone is in accord with the preferred formation of γ- over δ-lactones in the sugar series (10, pp. 33–34) and the hydroxylactones of stilbene-2-carboxylic acids (11).

The abnormal differences in the carbonyl stretching frequencies of samples of III*b* prepared as a potassium bromide disk and as a solution in chloroform may be due to

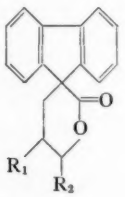
[1]*Manuscript received June 7, 1961.*
Contribution from the Chemistry Department, The University, Hull, East Yorkshire, England.
[2]*I.C.I. Fellow, 1958–60. Present address: Smith Kline & French Laboratories Ltd., Welwyn Garden City, Herts., England.*
**Commercial 3-chloropropanol contained approximately 20% of an isomeric chlorohydrin which was eliminated by careful fractional distillation.*



III

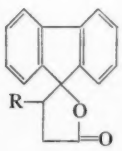
III	R_1	R_2	$\nu_{C=O}$, cm^{-1} $CHCl_3$	KBr	Δ
a	H	H	1773	1765	8
b	H	CH_2OH	1767	1735	32
c	H	C_6H_5	1772		
d	H	CH_2Cl	1788 (1770*)		
e	H	CH_2Br	1787		
f	H	CH_2I	1793		
g	H	$CHBrCH_3$	1778		
h	Br	$CHBr_2$	1805		

*Shoulder.

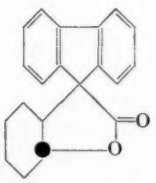


IV

IV	R_1	R_2	$\nu_{C=O}$, cm^{-1} $CHCl_3$	KBr	Δ
a	H	H	1730	1730	0
b	OH	H	—	—	
c	Br	CH_3		1732	
d	Br	C_6H_5	1748		



Va, R = H, $\nu_{C=O}$ (mull) = 1772 cm^{-1}
Vb, R = Br, $\nu_{C=O}$ (mull) = 1795 cm^{-1}



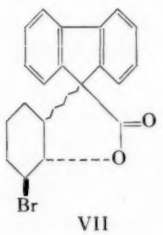
VI, $\nu_{C=O}$ ($CHCl_3$) = 1777 cm^{-1}

isomerization brought about by the high pressures required for disk formation (12, pp. 297–298; for another example of an abnormally low carbonyl frequency in a KBr disk, see ref. 13). Consequently, the infrared data discussed later in this paper refer to solutions in chloroform.

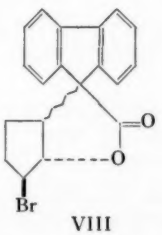
Reaction of the anion I*a* with *trans*-2-bromocyclohexanol, *cis*-2-chlorocyclohexanol, or cyclohexene oxide gave the same lactone (VI). Since cyclohexene oxide is known to react with nucleophiles with inversion at one carbon atom (14, 15), the lactone must have the trans structure. The lactone is formed from *trans*-2-bromocyclohexanol by prior formation of cyclohexene oxide (15) and from *cis*-2-chlorocyclohexanol by displacement with inversion (cf. the acetates of the 2-chlorocyclohexanols (16)). The reaction between I*a* and 2-chlorocyclohexanone failed, thwarting attempts to prepare the cis isomer of VI. Notwithstanding Westheimer's successful preparation of a hydroxy diester from cyclopentene oxide and diethyl malonate (14), the reaction between I*a* and *trans*-2-bromocyclopentanol failed to give the desired product.

The halolactonization and related reactions have recently aroused considerable interest. Van Tamelen and Shamma (17) have suggested that the formation of iodolactones is of diagnostic value in determining the position of unsaturation in olefinic acids, and this technique has been found to be of some use (18, 19). The formation of halolactones has proved particularly useful in the bicyclo[221]heptene series (e.g. ref. 20). The mechanism has been examined by Bartlett (21), Craig (22, 23), Arnold and co-workers (24, 25, 26, 27), and, in some detail, by Berti (9, 11, 28, 29, 30, 31). It has been shown to be stereospecific (9) and much evidence suggests that a carbonium ion or related species is the essential intermediate. Since α,α-disubstituted allylacetic acids form halolactones particularly readily (9), the opportunity has been taken to examine some 9-alkenylfluorene-9-carboxylic acids. (For the method of preparation of the acids, see ref. 3.)

9-Allylfluorene-9-carboxylic acid has now been shown to form a chloro-γ-lactone (III*d*) as well as the corresponding bromo (25) and iodo (24) compounds (III*e* and *f*, respectively). 9-Crotylfluorene-9-carboxylic acid* has been found to yield mainly the bromo-γ-lactone (III*g*) together with a trace of the isomeric δ-lactone (IV*c*), not obtained pure. 9-Cinnamylfluorene-9-carboxylic acid gave only the bromo-δ-lactone (IV*d*), which decomposed surprisingly readily to 9-cinnamylidenefluorene. The reactions of the crotyl and cinnamyl acids, like those of *cis*- and *trans*-stilbene-2-carboxylic acids (9), are best interpreted as proceeding by way of the more stable carbonium ion. It had been the intention to test this conclusion by examining the reactions of para-substituted cinnamyl derivatives but insufficient time has curtailed the project. 9-Propargylfluorene-9-carboxylic acid gave the heavily substituted tribromo-γ-lactone (III*h*), which decomposed at its melting point with evolution of bromine.



VII



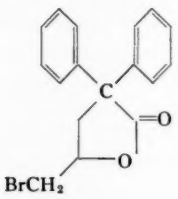
VIII

$\nu_{C=O}$ ($CHCl_3$) = 1777 cm^{-1} $\nu_{C=O}$ ($CHCl_3$) = 1777 cm^{-1}

**The structure of this acid was proved by hydrogenation of the methyl ester to the known (2) methyl 9-butylfluorene-9-carboxylate. This confirmation was necessary since crotyl bromide exists as an equilibrium mixture under normal conditions (32) and can give rise to 'abnormal products' by the S_N2 mechanism (33).*

9-3′-Cyclohexenylfluorene-9-carboxylic acid gave a good yield of the bromo-γ-lactone (VII). The homologous 3′-cyclopentenyl acid gave only a moderate yield of the lactone (VIII) but, nevertheless, afforded an example of the formation of a derivative of bicyclo-[330]octane under mild conditions. By analogy with Berti's studies (9), the lactones must have the partial stereochemistry shown. The preparation of VIII can be compared with the formation of the highly strained halo-β-lactones from dimethylmaleic and fumaric acids (21).

The carbonyl stretching frequency of the bromolactone prepared from allyldiphenylacetic acid (22) is 1783 cm^{-1}, showing it to be the γ-lactone IX.



IX

The infrared data in this paper show that introduction of halogen γ or δ to the carbonyl group of δ- and γ-lactones results in a shift of the carbonyl stretching frequency to higher frequencies, by 15–20 cm^{-1}. Similar results have only been reported previously for α-halo esters and α-halo ketones (12, pp. 443–509). The shift is shown by α-halo alicyclic ketones only when the halogen is equatorial and approximately coplanar with the carbonyl group. The larger shift of 32 cm^{-1} shown by the tribromolactone (III*h*) may be compared with the very exceptional shifts shown by esters of perfluoroalkyl carboxylic acids (34).

EXPERIMENTAL

Methyl fluorene-9-carboxylate was prepared and alkylated as described elsewhere (2, 3).

Lactone of 9-(2′-Hydroxyethyl)-fluorene-9-carboxylic acid (IIIa)

Methyl fluorene-9-carboxylate (0.7 g) and 2-bromoethanol gave the lactone, which crystallized from benzene as long white needles (0.5 g), m.p. 184–185°. Found: C, 82.56; H, 5.47%. Calculated for $C_{16}H_{12}O_2$: C, 82.73; H, 5.21%.

Reduction with ethereal lithium aluminum hydride gave an almost quantitative yield of 9-(2′-hydroxyethyl)-9-fluorenylmethanol, crystallizing from benzene–hexane as small colorless needles, m.p. 94–95°. Found: C, 80.0; H, 6.59%. Calculated for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71%.

Methyl fluorene-9-carboxylate and methyl bromoacetate gave methyl 9-carbomethoxymethylfluorene-9-carboxylate (88%), which formed colorless prisms from methanol, m.p. 91–92°. Found: C, 72.84; H, 5.60%. Calculated for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44%. Reduction with ethereal lithium aluminum hydride gave the diol described above, identity being established by mixed melting point determination and by comparison of infrared spectra.

Lactone of 9-(3′-Hydroxypropyl)-fluorene-9-carboxylic acid (IVa)

Methyl fluorene-9-carboxylate (0.7 g) and pure 3-chloropropanol (b.p. 69.5–70.0° at 19 mm; n_D^{24} 1.4487) gave the lactone, which formed colorless, well-defined prisms

(0.64 g) from benzene–hexane, m.p. 232–234°. Found: C, 81.69; H, 5.62%. Calculated for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64%.

γ-Lactone of 9-(2',3'-Dihydroxypropyl)-fluorene-9-carboxylic acid (IIIb)

Methyl fluorene-9-carboxylate and pure 2,3-dihydroxypropyl chloride gave the γ-lactone, which crystallized from chloroform–hexane as small colorless prisms (65–78%), m.p. 180–181°. Found: C, 76.85; H, 5.54%. Calculated for $C_{17}H_{14}O_3$: C, 76.67; H, 5.30%.

The infrared spectrum of a chloroform solution of the material retained in the mother liquors suggested the presence of methyl fluorene-9-carboxylate ($\nu_{C=O}$ 1760 cm^{-1}), further amounts of the γ-lactone (1770 cm^{-1}), and traces of the isomeric δ-lactone (1730 cm^{-1}).

Lactone of 9-(2'-Hydroxy-2'-phenylethyl)-fluorene-9-carboxylic acid (IIIc)

Methyl 9-ω-phenacylfluorene-9-carboxylate (6) (0.8 g) was reduced with a solution of sodium borohydride (0.5 g) in methanol (20 ml). The lactone separated from benzene–hexane as radiating clusters of elongated prisms (0.65 g), m.p. 180–181°, depressed to 165–171° by addition of the starting keto ester (m.p. 184–185°). Found: C, 84.49; H, 5.05%. Calculated for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16%.

Trans Lactone of 9-(2'-Hydroxycyclohexyl)-fluorene-9-carboxylic acid (VI)

Methyl fluorene-9-carboxylate and cyclohexene oxide gave the lactone, which separated from benzene–hexane as small, well-defined rhombs (88%), m.p. 272–273°. Found: C, 82.74; H, 6.21%. Calculated for $C_{20}H_{18}O_2$: C, 82.73; H, 6.28%.

Alkylation with *cis*-2-chlorocyclohexanol (15) or *trans*-2-bromocyclohexanol (prepared by a general method (35)) gave the same lactone, identified by mixed melting point determinations and by careful comparison of the infrared spectra of chloroform solutions. To obtain good yields of lactone from the bromohydrin it was found necessary to employ 2 moles of methoxide, indicating that the reaction proceeded by prior formation of cyclohexene oxide.

Several attempts to alkylate methyl fluorene-9-carboxylate with *trans*-2-bromocyclopentanol (prepared as for the cyclohexane homologue (35)) were unsuccessful. Occasionally traces of methyl 9-hydroxyfluorene-9-carboxylate were obtained as long white needles from cyclohexane, m.p. 160–161°, identified by comparison with an authentic specimen ((36), m.p. 158–159°).

Halolactones

The 9-alkenylfluorene-9-carboxylic acids were prepared by a general method (3). Methyl esters were characterized only when obtained crystalline. Bromolactones were prepared by adding a slight excess of a solution of bromine in chloroform to the appropriate acid.

9-Allylfluorene-9-carboxylic acid (37), most easily prepared according to reference 2, and the lactone of 9-(3'-bromo-2'-hydroxypropyl)-fluorene-9-carboxylic acid (25) have been described elsewhere. The lactone of 9-(3'-iodo-2'-hydroxypropyl)-fluorene-9-carboxylic acid (24) was more conveniently prepared by Van Tamelen's procedure (17).

The lactone of 9-(3'-chloro-2'-hydroxypropyl)-fluorene-9-carboxylic acid (III*d*), prepared by reaction of the acid with a solution of chlorine in chloroform (9), crystallized as needles from heptane (76%), m.p. 166–167°. Found: C, 71.76; H, 4.65%. Calculated for $C_{17}H_{13}O_2Cl$: C, 71.71; H, 4.60%. The same lactone was obtained in 64% yield from the reaction between thionyl chloride and the hydroxylactone (III*b*), using the conditions described by Berti (9).

The Lactone of 9-(3′-Bromo-2′-hydroxybutyl)-fluorene-9-carboxylic acid (IIIg)

9-Crotylfluorene-9-carboxylic acid was obtained as small colorless prisms (65%), m.p. 120–122°, after three crystallizations from benzene–hexane. Found: C, 81.99; H, 6.04%. Calculated for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10%. Esterification of the pure acid with diazomethane and hydrogenation of the oily methyl ester in methanol over 5% palladized charcoal gave methyl 9-butylfluorene-9-carboxylate, identical with an authentic specimen ((2), m.p. 34.0–34.5°). Saponification gave 9-butylfluorene-9-carboxylic acid, which crystallized as irregular rhombs from benzene, m.p. 115–116°. Found: C, 81.17; H, 6.81%. The identical acid was obtained by saponification of the authentic ester. The alternative product of hydrogenation, methyl 9-*s*-butylfluorene-9-carboxylate, has m.p. 70–71° (2).

The bromolactone separated from benzene–hexane as colorless needles (86%), m.p. 149–150°. Found: C, 63.06; H, 4.41%. Calculated for $C_{18}H_{15}O_2Br$: C, 63.00; H, 4.41%. Fractional crystallization of the mother liquors, using infrared spectra as a guide, gave a few milligrams of white needles, m.p. 112–116°, strongly depressed by the above lactone. The infrared spectra suggested that the minor product was the δ-lactone (IV*c*), originally present in approximately 1–4%.

The Lactone of 9-(2′,3′,3′-Tribromo-2′-hydroxypropyl)-fluorene-9-carboxylic acid (IIIh)

9-Propargylfluorene-9-carboxylic acid separated from benzene–hexane as colorless needles or prisms (72% based on methyl fluorene-9-carboxylate), m.p. 182–184° with decomposition. Found: C, 82.17; H, 4.85%. Calculated for $C_{17}H_{12}O_2$: C, 82.24; H, 4.87%. The methyl ester crystallized as colorless prisms from methanol, m.p. 117–119° (not analyzed). The infrared spectra of solutions of the acid and ester in chloroform showed the characteristic acetylenic C—H stretching band at 3300 cm^{-1} (8, p. 49).

The tribromolactone formed clusters of prisms (77%) from benzene–hexane, m.p. 155–156° with decomposition and evolution of bromine. Found: C, 42.05; H, 2.56%. Calculated for $C_{17}H_{11}O_2Br_3$: C, 41.92; H, 2.28%.

The Lactone of 9-(2′-Bromo-3′-hydroxy-3′-phenylpropyl)-fluorene-9-carboxylic acid (IVd)

9-Cinnamylfluorene-9-carboxylic acid crystallized from chloroform as well-defined prisms (71%), m.p. 162–163°. Found: C, 84.81; H, 5.72%. Calculated for $C_{23}H_{18}O_2$: C, 84.64; H, 5.56%. Hydrogenation in acetic acid over 10% palladium-on-charcoal gave 9-(3′-phenylpropyl)-fluorene-9-carboxylic acid, m.p. 124–126°, identical with an authentic specimen (38).

The crude bromolactone (1 g) was dissolved in cold chloroform (5 ml) and diluted with hot heptane (10 ml); pure lactone separated as small colorless prisms (0.6 g), m.p. 195–197° with decomposition to 9-cinnamylidenefluorene (below). Found: C, 68.24; H, 4.44; Br, 19.94%. Calculated for $C_{23}H_{17}O_2Br$: C, 68.16; H, 4.23; Br, 19.72%. The crude lactone was very unstable but samples of the pure substance have remained unchanged for 2 years.

The lactone (0.1 g) was boiled for 10 minutes with glacial acetic acid (2 ml). When the solution was cooled, 9-cinnamylidenefluorene separated as bright yellow needles (45 mg), m.p. 152–153° (lit. m.p. 150.5–151.0° for the all-trans isomer (39)).

The Lactone of 9-(3′-Bromo-2′-hydroxycyclohexyl)-fluorene-9-carboxylic acid (VII)

9-(3′-Cyclohexenyl)-fluorene-9-carboxylic acid, prepared from 3-bromocyclohexene (40), formed large, colorless prisms (73%) from benzene–hexane, m.p. 168–170° with decomposition. Found for a sample dried 12 hours at 80° and 1 mm; C, 82.52; H, 6.35%. Calculated for $C_{20}H_{18}O_2$: C, 82.73; H, 6.25%.

The bromolactone crystallized from benzene–hexane as colorless needles (89%), m.p. 186–187°. Found: C, 64.92; H, 4.70%. Calculated for $C_{20}H_{17}O_2Br$: C, 65.05; H, 4.64%.

The Lactone of 9-(3′-Bromo-2′-hydroxycyclopentyl)-fluorene-9-carboxylic acid (VIII)

Methyl 9-(3′-Cyclopentenyl)-fluorene-9-carboxylate

Alkylation of methyl fluorene-9-carboxylate with 3-chlorocyclopentene (41) gave a brown resin. Extraction with boiling heptane and passage of the resulting solution through a short column of activated alumina gave the pure ester, crystallizing from heptane as small colorless needles (67%), m.p. 93–94°. Found: C, 82.56; H, 6.32%. Calculated for $C_{20}H_{18}O_2$: C, 82.73; H, 6.25%. Hydrogenation in methanol over 5% palladized charcoal gave methyl 9-cyclopentylfluorene-9-carboxylate as elongated prisms from methanol or hexane, m.p. 83.0–84.5°. Found: C, 82.06; H, 6.78%. Calculated for $C_{20}H_{20}O_2$: C, 82.15; H, 6.89%. The same ester was obtained by alkylating fluorene ester with cyclopentyl bromide.

9-(3′-Cyclopentenyl)-fluorene-9-carboxylic acid separated from benzene–hexane as prismatic needles, m.p. 184–186°, after a change of crystalline form at 160–161°. Found: C, 83.12; H, 5.30%. Calculated for $C_{19}H_{16}O_2$: C, 82.78; H, 5.54%.

The bromolactone formed small prisms (45%) from benzene–hexane, m.p. 208–210°. Found: C, 64.17; H, 4.10%. Calculated for $C_{19}H_{15}O_2Br$: C, 64.24; H, 4.26%.

The Lactone of 5-Bromo-4-hydroxy-2,2-diphenylpentanoic acid (IX)

Allyldiphenylacetic acid was prepared as described (42) for methyldiphenylacetic acid. It formed rosettes of prisms (77%) from benzene–hexane, m.p. 143–144° (lit. m.p. 142–143° (43)). The lactone of 5-bromo-4-hydroxy-2,2-diphenylpentanoic acid (IX) crystallized as large prisms from benzene–hexane, m.p. 88–89° (lit. m.p. 87–88° (22)). Found: C, 62.03; H, 4.39%. Calculated for $C_{17}H_{15}O_2Br$: C, 61.64; H, 4.57%.

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PYRROLES RELATED TO PORPHOBILINOGEN[1]

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ABSTRACT

A study of the decarboxylation of pyrroles related to 5-carboxyporphobilinogen has led to an improved synthesis of porphobilinogen, to isoporphobilinogen, and to other pyrroles required for the preparation of possible intermediates in the biosynthesis of porphyrins.

Among the porphobilinogen derivatives which have been considered as possible intermediates in the biosynthesis of uroporphinogens from porphobilinogen are isoporphobilinogen, 5-aminomethylporphobilinogen (2), the carbinols analogous to these and to porphobilinogen, and polypyrranes. The behavior of synthetic specimens of these with enzymes is pertinent to the biogenetic problem. Also, a study of isoporphobilinogen under conditions which convert porphobilinogen to uroporphinogens might clarify ill-defined assumptions relating the mechanisms of the enzymic and non-enzymic conversions of porphobilinogen.

Many projected syntheses of these postulated intermediates required pyrroles with one α-position free and the other bearing a potential aminomethyl group, carbinol group, or bridge. We had found only one route to such intermediates in the decarboxylation of 5-carboxyporphobilinogen lactam in boiling water, a method too dependent on structure for its applicability in analogous cases to be assessed other than empirically. Consequently, other methods were studied for decarboxylating 5-carboxypyrroles with appropriate substituents in the remaining positions.

The triethyl ester of I*a* had been converted into the ester of I*c* and thence into I*c* itself (3). This last was treated with iodine in bicarbonate to yield the iodo-aldehyde I*f*. Although this product was obtained only once analytically pure, the other specimens were otherwise undistinguished and were quite satisfactory as intermediates. The analytical difficulties could not be ascribed to an iodine–pyrrole complex (4). The iodo-aldehyde I*f* was reduced catalytically to the α-free aldehyde I*h*. The carbinol (I, R′ = H, R″ = CH_2OH) would be expected to be very unstable (5) and has not yet been isolated as a reduction product of I*h*. The oxime, I*i*, was obtained from I*h* but more conveniently by two unexpected routes. An attempt to prepare the oxime of I*f* led directly to I*i*, iodine being lost; the ready dehalogenation of some iodopyrroles has been reported (4). This dehalogenation with hydroxylamine is of limited applicability as the triethyl ester of I*g* survived the same treatment unchanged. An attempt to improve the conversion of the aldehyde I*c* into its oxime I*d* (3) led to the third and best preparation of I*i*: longer heating resulted in the expected oxime I*d* being decarboxylated to I*i*. The ease of this decarboxylation may be unrelated to that of 5-carboxy-alkylpyrroles and of 5-carboxy-porphobilinogen lactam; some 5-carboxypyrromethenes also decarboxylate readily (6).

Under carefully chosen conditions (see below and Experimental) the catalytic reduction of I*i* gave as the sole product porphobilinogen I*j*, identified by comparison with natural material as such, as the hydrochloride, and as the lactam. The yield of porphobilinogen from the triethyl ester of I*a* in four steps was 29%. On a much smaller scale our previous synthesis (7) had given 5% in seven steps after extensive purification.

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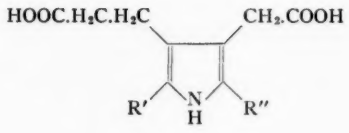

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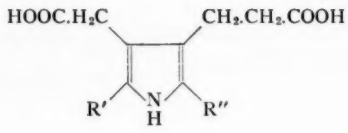
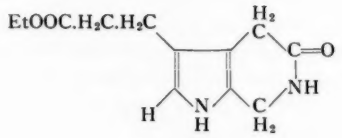
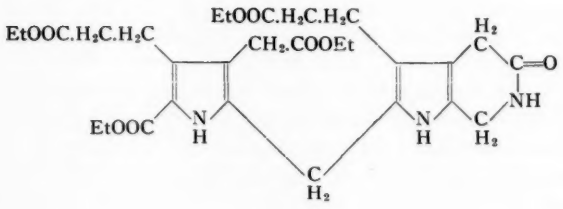


II

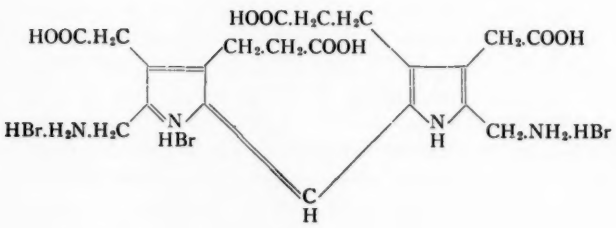
(*a*) R′ = COOH, R″ = CH_3
(*b*) R′ = COOH, R″ = CH_2Br
(*c*) R′ = COOH, R″ = CHO
(*d*) R′ = COOH, R″ = CH=N.OH
(*e*) R′ = COOH, R″ = $CH_2.NH_2$
(*f*) R′ = I, R″ = CHO
(*g*) R′ = I, R″ = COOH
(*h*) R′ = H, R″ = CHO
(*i*) R′ = H, R″ = CH=N.OH
(*j*) R′ = H, R″ = $CH_2.NH_2$
(*k*) R′ = H, R″ = CN
(*l*) R′ = COOH, R″ = H
(*m*) R′ = H, R″ = CH=C(CN)COOEt
(*n*) R′ = CHO, R″ = CN
(*o*) R′ = CHO, R″ = CH=C(CN)COOEt



III



IV



V

The carbinol (I, R′ = H, R″ = CH_2OH) was mentioned above, and routes to aminomethyl-porphobilinogen (I, R′ = R″ = CH_2NH_2) are also being explored. When the aldehyde group of I*h* was protected by condensation with cyanoacetic ester (8), the product, I*m*, could not be esterified. However, the aldehyde group of the ethyl ester of I*h* was similarly

protected and the resulting ester of I*m* was formylated to the ethyl ester of I*o* using dimethyl formamide and phosphorus oxychloride. The methyl ester of the oxime I*i*, which formed a stable hydrochloride, was converted into the methyl ester of the nitrile I*k* by dimethyl formamide and phosphorus oxychloride under mild conditions. Under more vigorous conditions the same reagents formylated the nitrile ester I*k* to the ester of the aldehyde-nitrile I*n*.

The structures of I*k* and I*n* were confirmed by conversion into known derivatives of II*a*: I*k* into II*l* and its ethyl ester, I*n* into II*c*. The sequence implies a formally general solution to the problem of interconverting isomeric 3,4-dialkylpyrroles such as I*a* and II*a*. Only particular solutions had been found to this problem which was once of some practical significance (9).

Both porphobilinogen and isoporphobilinogen (I*j* and II*j*) had been identified by paper chromatography among the products obtained by heating their 5-carboxy derivatives (I*e* and II*e*) with copper acetate in aqueous pyridine (10). However, neither had been isolated and in the case of porphobilinogen, our own experience with this synthesis had been discouraging (7). Accordingly, the synthesis of isoporphobilinogen originally projected depended on the decarboxylation of II*e* after acylating the primary amino group, a closer analogue of the easily decarboxylated 5-carboxyporphobilinogen lactam being hardly to be expected. To this end the ethyl ester of the aldehyde II*c*, obtained from II*a*, was converted to the ester of the oxime II*d* and thence to the ethyl ester hydrochloride of II*e*. We then turned to the following more attractive route analogous to the new synthesis of porphobilinogen. The aldehyde II*c*, obtained from its ester, was converted into isoporphobilinogen through II*f* and II*i*. We also converted II*f* into the α'-free aldehyde II*h*.

The reduction of the oxime I*i* to porphobilinogen, I*j*, had been carried out in relatively dilute solution to completely suppress the formation of a by-product of unknown structure and higher R_f (see Experimental). Although both products showed some reactions expected of porphobilinogen and gave similar analytical figures, the required product was identified through derivatives as a monohydrate corresponding to the structure I*j*, and through comparison with natural material, as porphobilinogen.

The reduction of the oxime II*i* to isoporphobilinogen, II*j*, had to be carried out at greater dilution than was that of I*i*, to completely suppress the formation of small amounts of an analogous by-product which again had a higher R_f. Isoporphobilinogen could be identified because it analyzed for anhydrous II*j*, had an R_f identical with that of porphobilinogen, and gave a well-characterized diethyl ester hydrochloride.

Of the pyrromethanes related to porphobilinogen, only the 5,5′-free compounds are known (11). In two approaches to others, the ethyl ester of porphobilinogen lactam, III, was condensed with the ester of I*b*, and 5-carboxyporphobilinogen, I*e*, was condensed with formic acid and hydrogen bromide to give compounds which may be provisionally formulated as IV and V, although we have not yet developed methods for checking the homogeneity of such pyrromethanes and pyrromethenes by paper chromatography. The methene V was reduced to a colorless substance which did not analyze well for the corresponding pyrromethane and which was not converted into uroporphyrins enzymically (12).

EXPERIMENTAL

The melting points are uncorrected as taken in capillaries. The infrared spectra in Nujol mull and X-ray powder photographs are by Dr. R. N. Jones and Mr. R. Lauzon,

and by Dr. Maria Przybylska. Paper chromatograms were ascending overnight runs in ethanol – concentrated ammonia – water (7:1:2), using an acidic permanganate spray (13) or, in the upper layer of *n*-butanol – glacial acetic acid – water (4:1:5), using an Ehrlich's spray. Such runs determining R_f's designated by "in ammonia" and "in acetic acid" respectively showed one spot only. Darco refers to Darco G-60 charcoal and ether, to anhydrous ether. The analyses on specimens dried to constant weight were by Mr. J. R. H. Séguin.

2-Formyl-5-carboxy-pyrrole-3-acetic acid-4-propionic acid (Ic)

The following method has given more consistent yields than did the earlier method (3). The triethyl ester of Ic (16 g) (3) and 360 ml of absolute ethanol were added to 24 g of sodium hydroxide in 600 ml of water. Nitrogen was passed through while the mixture was warmed to solution and then kept at 50° for 1 hour. The orange solution was then concentrated to 100 ml in a rotary evaporator at <40°. The red concentrate was maintained below 10° while it was acidified to Congo red with 52 ml of concentrated hydrochloric acid. The temperature was then raised to 25°. The product crystallized as brownish-pink microneedles (12 g) which were filtered off after ¼ hour and washed with water, then with ether. It was purified by extracting into 800 ml of acetone (Soxhlet, 5 hours, 200 mg insoluble). The cooled acetone solution was forced by air pressure through a column (35 mm diam. × 60 mm of Darco/Celite 535 (1:1 by vol.) supported on a 15-mm bed of Celite 535) which was then thoroughly washed with acetone. When the pale yellow eluate was concentrated to 75 ml and cooled to 0°, 9.9 g (81%) of colorless needles separated; m.p. 241–243° (decomp., inserting at 235°), Ehrlich test dark red on heating; R_f "in ammonia": 0.19.

For analysis the product was recrystallized from acetone (Soxhlet) and dried at 56° and 5×10^{-2} mm. Calc. for $C_{11}H_{11}O_7N$: C, 49.07; H, 4.12; N, 5.20. Found: C, 49.33; H, 4.24; N, 5.32.

2-Formyl-5-iodopyrrole-3-acetic acid-4-propionic acid (If)

A suspension of 4.0 g of Ic in 60 ml of water and 3.0 g of $KHCO_3$ was added to a solution of 9.0 g KI and 3.8 g of iodine in 60 ml of water. After the mixture was shaken to solution on the steam bath, heating was continued for 1 hour while carbon dioxide was evolved and the color lightened considerably. A cold finger, occasionally washed down with a few drops of absolute ethanol, prevented the loss of iodine. The solution was cooled to 20° and scratched. After ¼ hour the crude product (2.9 g, m.p. 196–197° decomp.) was filtered off and washed with water. After two recrystallizations from 65 ml of water, flesh-colored needles resulted (2.6 g, 50%); m.p. 199–200° (decomp., iodine evolved, inserted at 185°); Ehrlich test slowly positive cold; R_f "in ammonia": 0.40.

For analysis it was dried (60°, 1×10^{-4} mm). Calc. for $C_{10}H_{10}O_5NI$: C, 34.21; H, 2.87; N, 3.99; I, 36.15. Found: C, 34.28; H, 2.94; N, 4.17; I, 36.47, 36.04 (Schöniger), 35.97 (Carius).

2-Formylpyrrole-3-acetic acid-4-propionic acid (Ih)

The iodopyrrole If (2.0 g) in 60 ml of absolute ethanol was added to 25 ml of water containing 776 mg of sodium acetate trihydrate and 250 mg of palladium black. The mixture was shaken under hydrogen (20°, 1 atm) until absorption ceased after the uptake of 1 mole during 1 hour. The filtrate and aqueous washings were taken to dryness in a rotary evaporator (bath at 50°) and 20 ml of water was twice added and removed in the same way. The residue was extracted with 5 ml of water and the insoluble product

filtered off and then washed (2×1 ml). This product was dried and then extracted into 200 ml of ether (Soxhlet, 6 hours). After the ether solution had been concentrated to 50 ml, yellow needles separated (970 mg, 76%); m.p. 176–180° (decomp., inserting at 160°); Beilstein test for halogen negative; Ehrlich test (magenta) fast in the cold; R_f "in ammonia": 0.33; in ethanol, λ_{max} 302 mμ ($\epsilon = 16\times10^3$). The aldehyde CH and the pyrrole ring CH were both confirmed by the N.M.R. spectrum.*

For analysis the product in acetone was decolorized by passage through a small column of Darco. The precipitate obtained from the eluate by adding ether was recrystallized three times from acetone–ether: colorless, flat needles, m.p. unchanged. Calc. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.61; H, 4.94; N, 6.12.

2-Formylpyrrole-3-acetic acid-4-propionic acid Diethyl Ester

The aldehyde I*h* (351 mg) was warmed to solution in 5 ml of absolute ethanol, and diazoethane (from 3 g of nitrosoethylurea) in 75 ml of ether was added to the cooled solution. Evaporation left an oil which was dried *in vacuo* overnight and then crystallized by rubbing with ether. After sublimation (135°, 5×10^{-4} mm) it was obtained as pale yellow crystals (353 mg, 79%); m.p. 47–52°, softening from 37°; Ehrlich test red in the cold. The product of only one run crystallized and was not analyzed.

2-Formylpyrrole-3-acetic acid-4-propionic acid Oxime (Ii)

Method (a)

Hydroxylamine hydrochloride (500 mg) was dissolved in 3 ml of water, and 2 ml of 10% sodium hydroxide was added. The aldehyde I*h* (128 mg) and 1.4 ml of this solution were heated on the steam bath for ½ hour. The cooled solution was acidified to Congo red with 2 drops of concentrated hydrochloric acid and cooled at 0° for ½ hour; then the product (128 mg, 94%) was separated and washed successively with water, ethanol, and ether. It formed colorless microcrystals; m.p. 210–212° (decomp., inserted at 200°); Ehrlich test slowly violet cold. The R_f "in ammonia" (0.29) and the X-ray powder photograph were identical with those of the products of methods (*b*) and (*c*) below.

For analysis it was recrystallized four times (70% recovery) from 0.01 *N* NaOH/0.01 *N* HCl and dried at 60°, 0.02 mm. Calc. for $C_{10}H_{12}O_5N_2$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.82; H, 5.11; N, 11.55.

Method (b)

The iodopyrrole I*f* (3 g), 6 g of hydroxylamine hydrochloride, 36 ml of water, and 24 ml of 10% sodium hydroxide were heated for 1 hour on the steam bath (gases evolved). The cooled solution was passed through a column of Darco (22 mm diam.×10 mm), the column being washed with 15 ml of water. The yellow filtrate and washings were made acid to Congo red with ca. 18 ml of 2 *N* HCl, then cooled to 0° for 1 hour. The product (1.7 g, 81%) was separated and washed successively with water, ethanol, and ether to give cream-colored crystals; m.p. 212–213.5° (decomp., inserted at 200°); Beilstein halogen test negative, Ehrlich test slowly violet cold; in ethanol, λ_{max} 287 mμ ($\epsilon = 19\times10^3$).

For analysis it was recrystallized from acetone (Soxhlet) and dried (60°, 0.1 mm), giving cream-colored needles, m.p. 211–212° (decomp., inserted at 200°). Found: C, 49.89; H, 5.05; N, 11.51.

Preparative Method (c)

Hydroxylamine hydrochloride (24 g) in 144 ml of water was brought to pH 6–7 with

*We are grateful to Dr. H. J. Bernstein for this interpretation.

96 ml of 10% NaOH. The 5-carboxylpyrrole I*c* (9 g) was added and the solution was refluxed (mantle) under nitrogen for 7 hours in the dark. After being left at 20° in the dark overnight (important), the solution was filtered through a column of Darco (18 mm diam. × 40 mm, prewashed with water) and the column washed with water until the eluate was colorless. The purple eluate was acidified to Congo red with ca. 95 ml of 2 *N* HCl, scratched, and left at 0° for 1½ hours. The nearly colorless diamond-shaped crystals (6.02 g) were separated and washed successively with water, ethanol, and ether. A colorless product (5.29 g, 66%) was obtained after two recrystallizations from 0.1 *N* NaOH/2 *N* HCl (to pH 3); m.p. 210–211° (decomp., inserted at 200°); R_f "in acetic acid": 0.67.

For analysis it was recrystallized twice more in the same way and dried (60°, 0.01 mm). Found: C, 49.85; H, 5.33; N, 11.80.

2-Formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester Oxime

A solution of 2 g of the oxime I*i* in 60 ml of 5% hydrogen chloride in methanol was kept at 0° overnight and then taken to dryness *in vacuo* below 20° in a rotary evaporator. To the residue dissolved in 100 ml of water, 4 ml of 2 *N* NaOH was added. After ½ hour the red-brown precipitate was separated, washed with water, dried (weight: 2 g), and extracted into 750 ml of ether (Soxhlet, 2 hours). The light brown extract was filtered through a column of Darco (18 mm diam. × 16 mm). The colorless eluate and ether wash were concentrated to 15 ml, and 50 ml of *n*-pentane was added with scratching. The colorless needles which separated (1.86 g, 83%) were washed with pentane; m.p. 130–133°; Beilstein halogen test negative; Ehrlich test slowly red cold; in ethanol, λ_{max} 285 mμ ($\epsilon = 20\times10^3$).

For analysis it was recrystallized twice from methanol–water (65% recovery) and dried (20°, 0.01 mm), m.p. 131–135°. Calc. for $C_{12}H_{16}O_5N_2$: C, 53.72; H, 6.01; N, 10.44; OMe, 23.14. Found: C, 53.86; H, 5.84; N, 10.53; OMe, 22.88.

2-Formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester Oxime Hydrochloride

A solution of 201 mg of the oxime I*i* in 6 ml of 5% hydrogen chloride in methanol was kept at 0° for 3 hours and then taken to dryness at 20°; 6 ml of methanol was thrice added and removed in the same way. The residue was slurried with 10 ml of chloroform and the pink crystals (181 mg, 71%) filtered off, m.p. 160–164° (decomp., inserted at 150°); Beilstein halogen test positive; Ehrlich test very slowly violet cold.

For analysis it was twice precipitated from methanol by ether at 20° and dried (25°, 1×10^{-4} mm), giving colorless needles (35% recovery), m.p. 162–164° (decomp., inserted at 150°). Calc. for $C_{12}H_{16}O_5N_2$.HCl: C, 47.29; H, 5.62; N, 9.19; Cl, 11.64; OMe, 20.37. Found: C, 47.43; H, 5.65; N, 9.05; Cl, 11.44; OMe, 20.22.

2-Aminomethylpyrrole-3-acetic acid-4-propionic acid (Porphobilinogen, Ij)

A suspension of 5 g of the oxime I*i* and 2 g of palladium black in 100 ml of water was shaken under hydrogen at room temperature and pressure until absorption ceased after the uptake of 2 moles in about 2 hours. After the addition of 5 ml of concentrated ammonia to dissolve the product, filtration through paper (Whatman No. 5) gave a yellow solution. After bringing its pH to 7 with glacial acetic acid this solution was filtered through a column of alumina (Woelm, neutral, prewashed with water, 18 mm diam. × 50 mm) and the column washed with water until the Ehrlich reaction of the eluate was faint. The eluate (400 ml) was concentrated to 100 ml in a rotary evaporator (oil pump, baths 25–30° and ice–salt). The concentrate was acidified to pH 4.5 with glacial acetic acid with scratching, quickly cooled, and kept at 0° for 4 hours. The product, after being washed successively with water, ethanol, and ether, formed buff-colored, barrel-shaped

plates (3.2 g, 63% as the monohydrate); m.p. 174–177° (decomp., inserting at 167°); Ehrlich reaction rapidly positive cold. Its R_f "in acetic acid" (0.49) and its infrared mull spectrum were identical with those of natural porphobilinogen.

The X-ray powder photograph was identical with that of porphobilinogen synthesized over the lactam (7). Between these photographs and that of natural material there were differences in the relative intensities of 3 lines among 30. These differences are not considered significant because the natural porphobilinogen was photographed only as received, when its infrared mull spectrum showed a gross anomaly, unlike the spectrum of the same material after recrystallization as the hydrochloride and then as the base.

The synthetic porphobilinogen also agreed with natural material in the following respects:* (1) the quantitative Ehrlich reaction; (2) paper electrophoresis at pH 2.4, 4.4, 6.6, and 10.4; (3) the uroporphyrin mixtures (examined by paper chromatography after conversion to coproporphyrins) formed by the self-condensation of the porphobilinogen at various pH's; (4) the enzymic conversion to uroporphyrin.

For analysis it was recrystallized from 0.7 *N* ammonia – glacial acetic acid and dried (20°, 0.01 mm). Calc. for $C_{10}H_{14}O_4N_2.H_2O$: C, 49.17; H, 6.60; N, 11.47. Found: C, 48.88; H, 6.87; N, 11.41.

Porphobilinogen Lactam

This synthetic porphobilinogen was converted into the lactam (52%) by the method of Cookson and Rimington (14) as colorless hexagonal plates, Ehrlich test fast cold.

For analysis it was recrystallized from water (Soxhlet) and dried (20°, 0.01 mm), m.p. 281–283° (decomp., inserted at 270°). It was identical with the lactam from natural porphobilinogen by the following criteria: infrared mull spectrum, X-ray powder photograph, and R_f (0.63 "in acetic acid"). Calc. for $C_{10}H_{12}O_3N_2$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.43; H, 5.77; N, 13.35.

Porphobilinogen Hydrochloride

This synthetic porphobilinogen was converted into the monohydrate of the hydrochloride (59%) by the method of Cookson and Rimington (14).

For analysis it was recrystallized from 2 *N* hydrochloric acid, dried (2 hours, 20°, 0.01 mm), and then exposed to air overnight to give faintly pink microneedles giving an I.R. mull spectrum and X-ray powder photograph identical with those of the natural hydrochloride. Calc. for $C_{10}H_{14}O_4N_2.HCl.H_2O$: C, 42.78; H, 6.10; N, 9.98; Cl, 12.63. Found: C, 43.01; H, 5.98; N, 9.89; Cl, 12.44.

A *by-product* of the reduction of the oxime I*i* to porphobilinogen was initially very troublesome. The formation of this by-product, like that of porphobilinogen, was accompanied by the uptake of 2 moles of hydrogen. The ratio of the two products was independent of the hydrogen pressure but the by-product was favored by higher oxime concentrations and by reduction in dilute ammonia rather than in water. For example, 100 mg of the oxime with the catalyst in 1 ml of 1.5 *N* ammonia (1 atm of hydrogen) gave the by-product and a trace of porphobilinogen; in 25 ml of 0.7 *N* ammonia (1 atm or 120 atm of hydrogen) or in 1 ml of 0.7 *N* acetic acid (1 atm of hydrogen), porphobilinogen and a trace of by-product resulted.

The by-product analyzed much like the porphobilinogen monohydrate, gave a positive Ehrlich test cold, gave uroporphyrins with hot 2 *N* hydrochloric acid, and formed a hydrochloride. However, it gave no lactam by the method of Cookson and Rimington (14)

*We are indebted to Dr. J. J. Scott and to Professor L. Bogorad for the tests with enzymes, and to Dr. D. Mauzerall for the other tests.

and it differed from porphobilinogen in its R_f (0.66 "in acetic acid"), in its X-ray powder photograph, and in the infrared mull spectra of both the base and hydrochloride.

For analysis it was recrystallized from 1.5 *N* ammonia – acetic acid and dried (20°, 1×10^{-4} mm) to give colorless hair-like microcrystals, m.p. 178–181° (decomp., inserted at 166°). Calc. for $C_{10}H_{14}O_5N_2$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.68; H. 6.31; N, 11.05.

2-(ω-Cyano-ω-carbethoxy-vinyl)-pyrrole-3-acetic acid-4-propionic acid Diethyl Ester (Ethyl Ester of Im)

The ethyl ester of the aldehyde I*h* (the crude oily product from 351 mg of I*h* and ethereal diazoethane) was heated for ½ hour on the steam bath with 200 mg of ethyl cyanoacetate, 5 ml of absolute ethanol, and 2 drops of 25% aqueous methylamine. The crude product, which separated on cooling and scratching, was recrystallized from ethanol, giving 297 mg (51%), m.p. 108–110°.

For analysis it was recrystallized four times from ethanol (80% recovery) and dried (60°, 0.01 mm), giving yellow microneedles; m.p. 111–112° (softening from 105°); Ehrlich's test blue hot; insoluble in cold ethanol. Calc. for $C_{19}H_{24}O_6N_2$: C, 60.62; H, 6.43; N, 7.44. Found: C, 60.91; H, 6.50; N, 7.34.

The acid I*m* was prepared analogously from I*h* using aniline as the catalyst. It formed yellow crystals from ethanol; m.p. 191–194° (decomp., inserted at 180°); soluble in hot acetone; insoluble in refluxing ether. Attempts to esterify it with 5% ethanolic hydrogen chloride and with ethereal diazoethane failed.

2-(ω-Cyano-ω-carbethoxy-vinyl)-5-formylpyrrole-3-acetic acid-4-propionic acid Diethyl Ester (Ethyl Ester of Io)

The ethyl ester of I*m* (247 mg), suspended in 0.428 ml of dimethyl formamide, was treated with 0.070 ml of phosphorous oxychloride (10% excess) and heated to solution on the steam bath, then for 10 minutes longer. Water (15 ml) containing 573 mg of sodium acetate trihydrate was added to the cooled solution. After the mixture was scratched and allowed to stand ½ hour, the brownish-yellow product separated. Its solution in 10 ml of chloroform was filtered through a column of alumina (Woelm neutral grade IV, 8 mm diam. × 20 mm). The residue left on evaporating the eluate was recrystallized from ether – *n*-pentane and then from ethanol–water, giving yellow needles (137 mg, 52%), m.p. 107–109°.

For analysis it was twice recrystallized from ethanol–water (65% recovery) and dried (56°, 5×10^{-4} mm), m.p. 110–111°. Calc. for $C_{20}H_{24}O_7N_2$: C, 59.40; H, 5.98; N, 6.93. Found: C, 59.35; H, 5.79; N, 6.99.

2-Cyanopyrrole-3-acetic acid-4-propionic acid Dimethyl Ester (Methyl Ester of Ik)

The dimethyl ester of I*i* (500 mg, 1.87 mmoles) was warmed to solution in 0.67 ml of dimethyl formamide (previously distilled over phosphorus pentoxide). This solution was shaken vigorously at −15° while 0.19 ml (2.06 mmoles) of phosphorus oxychloride was added slowly, then left at 20° for ¼ hour. The mixture was treated with 1.125 g (8.3 mmoles) of sodium acetate trihydrate in 5 ml of water, diluted to 25 ml with water, and scratched; light brown crystals (394 mg) then separated. These were extracted into 200 ml of ether (Soxhlet, 1 hour), the solution was filtered through a column of Darco (8 mm diam. × 15 mm), and the column was washed with ether. The colorless eluate was concentrated to 10 ml. Crystallization was induced by scratching and completed by evaporating more ether while adding *n*-pentane. The product (344 mg, 74%) formed colorless

glittering crystals, Ehrlich test faintly rose hot. It exists in two forms, m.p. 81–83° and 103–106°, either of which might separate from solution or melt and which gave identical infrared mull spectra (nitrile band at 2240 cm^{-1}). The U.V. spectrum in ethanol, λ_{max} at 256 mμ ($\epsilon = 12\times10^3$) and 235 mμ ($\epsilon = 8\times10^3$), with no absorption at 280–290 mμ, showed that the oxime was absent.

For analysis it was twice recrystallized from ether – *n*-pentane (74% recovery) and dried (20°, 0.01 mm), m.p. 80–82° (softening from 75°). Calc. for $C_{12}H_{14}O_4N_2$: C, 57.59; H, 5.64; N, 11.20; OMe, 24.80. Found: C, 57.40; H, 5.62; N, 11.35; OMe, 24.64.

2-Carboxypyrrole-3-acetic acid-4-propionic acid (IIl)

The dimethyl ester of the nitrile I*k* (102 mg) in 4 ml of 10% sodium hydroxide and enough ethanol (about 2 ml) to effect solution was heated on the steam bath for 6 hours while nitrogen was bubbled through it and its volume maintained at 4 ml by the occasional addition of water. The cooled solution was passed through a column of Amberlite IR-120 (wet hydrogen form, 16 g) and the column was washed with water until the eluate gave a negative Ehrlich test cold. The residue left on taking the eluate to dryness in a rotary evaporator (bath at 45°) was extracted by 5×15-ml portions of boiling acetone. The extract was filtered and concentrated to 5 ml, 20 ml of ether was added, and amorphous mauve-colored impurities were filtered off at once. When the filtrate was scratched, the product (55 mg, 56%) crystallized as nearly colorless rectangular plates; m.p. 183–184° (decomp., inserted at 165°), undepressed by authentic material derived from the ester of II*a* (lit. 178° (3)); Ehrlich test quickly bluish-red cold. The R_f "in ammonia" (0.16) was identical with that of authentic material.

2-Carboxypyrrole-3-acetic acid-4-propionic acid Triethyl Ester

The acid (from the ester of I*k*) with diazoethane in ether gave the product as colorless needles; Ehrlich test slowly bluish-red cold; m.p. 51–52.5°, undepressed by authentic material (lit. 51–52° (3)). The two specimens also gave identical infrared mull spectra and X-ray powder photographs.

2-Cyano-5-formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester (Ester of In)

The ester of the nitrile I*k* (200 mg, 0.8 mmole) was dissolved in 0.4 ml of dimethyl formamide (previously distilled over phosphorus pentoxide), and 0.1 ml (1.1 mmole) of phosphorus oxychloride was added. The solution was heated for 25 minutes on the steam bath in an open vessel and cooled to 20°, and 592 mg (4.4 mmoles) of sodium acetate trihydrate in 2.5 ml of water was added. The suspended oil crystallized after the solution was scratched. After the volume of the mixture was made up to 8 ml with water, the buff-colored precipitate was filtered off and washed with water. The dried precipitate (134 mg) was dissolved in 125 ml of boiling ether and the cooled solution filtered through a column of Darco (8 mm diam. × 10 mm). The colorless eluate was concentrated to 2 ml, crystallization was induced by scratching, and the product (112 mg, 50%) was filtered off and washed with 1 ml of ether and then with *n*-pentane. It formed colorless plates; m.p. 113–116° (softening from 90°); Ehrlich test slowly brown hot; infrared band at 2255 cm^{-1} in Nujol mull; in ethanol, λ_{max} 294 mμ ($\epsilon = 19\times10^3$). It can be distilled at 160°, 1×10^{-5} mm.

For analysis it was recrystallized from ether and twice dried (20° and 1×10^{-2} mm, then 100° and 1×10^{-3} mm), giving colorless plates, m.p. 113–115°. Calc. for $C_{13}H_{14}O_6N_2$: C, 56.11; H, 5.07; N, 10.07. Found: C, 55.87; H, 4.98; N, 9.99. Specimens dried at 20°, softened from about 100°, and analyzed as a hydrate containing $\frac{1}{3}H_2O$.

2-Carboxy-5-formylpyrrole-3-acetic acid-4-propionic acid (IIc)

(a) From the Triethyl Ester of Ia via the Dimethyl Ester of In

This was carried out before the ester of I*n* had been obtained crystalline. The crude oily ester of I*n* (obtained as above by adding aqueous sodium acetate after reacting the ester of I*k* with dimethyl formamide and phosphorus oxychloride) was hydrolyzed for 6 hours on the steam bath in 10% sodium hydroxide. After passage through Amberlite IR-120 (hydrogen form), the solution was evaporated to dryness. The solution of the residue in acetone was decolorized by filtration through a column of Darco and was then taken to dryness. Adding ether to the residue induced the product to crystallize. This showed a single spot on paper chromatography and gave an infrared mull spectrum, X-ray powder photograph, and R_f ("in ammonia" but descending for 70 hours to ensure the absence of II*l*) identical with those of authentic material obtained ultimately from the ester of II*a* by method (*b*) below.

(b) Preparative Method (from the Triethyl Ester of IIa via the Triethyl Ester of IIc)

The triethyl ester of II*c* (3) (16 g) was hydrolyzed to II*c* just as the ester of I*c* was to I*c*. The crude product (red crystals, 9.36 g) was extracted into acetone and passed through Darco to give 7.34 g (60%) of cream-colored crystals; m.p. 234–238° (decomp., inserted at 225°); Ehrlich test slowly brownish-red cold; R_f "in ammonia" identical with that of I*c*.

For analysis it was recrystallized from acetone (Soxhlet) and then at <50° from water and then dried at 25° and 1×10^{-2} mm to give pink radiating needles. Calc. for $C_{11}H_{11}O_7N$: C, 49.07; H, 4.12; N, 5.20. Found: C, 48.97; H, 4.38; N, 5.14.

2-Formyl-5-iodopyrrole-3-propionic acid-4-acetic acid (IIf)

The preparation of II*f* from 4 g of II*c* followed that of I*f* from I*c*. The buff-colored crystalline crude product (1.89 g), m.p. 221–222° (decomp., introduced at 200°), was twice recrystallized from 110 ml of water. The product (1.46 g, 28%) formed yellow needles; m.p. 221–224° (decomp., iodine evolved, introduced at 200°); Ehrlich test slowly positive cold; R_f "in ammonia" identical with that of I*f*.

For analysis an acetone solution was decolorized by filtration through a small column of Darco. The precipitate, obtained from the eluate by adding hexane, was recrystallized four times from water and dried (56°, 1×10^{-4} mm), giving colorless needles, m.p. 215–220° (decomp., iodine evolved, introduced at 200°). Calc. for $C_{10}H_{10}O_5NI$: C, 34.21; H, 2.87; N, 3.99; I, 36.15. Found: C, 34.35; H, 3.12; N, 3.99; I, 36.24.

2-Formylpyrrole-3-propionic acid-4-acetic acid (IIh)

The iodopyrrole II*f* (500 mg) in 24 ml absolute ethanol was added to 194 mg sodium acetate trihydrate in 16 ml of water, and the solution was shaken for 1 hour under hydrogen (20°, 1 atm) with 125 mg of palladium black. The filtrate and aqueous washings from the catalyst were taken to dryness in a rotary evaporator (bath temp. 45°), then 5 ml of water was twice added and removed in the same way. The residue was extracted into 75 ml of ether (Soxhlet, 6 hours). The residue left on evaporating the ether was dissolved in 15 ml of boiling acetone. This solution was filtered through a column of Darco, the eluate concentrated to 5 ml, and the colorless product (175 mg, 55%) precipitated by the addition of 20 ml of ether.

For analysis it was recrystallized twice from water (56% recovery) and dried (56°, 1×10^{-4} mm) to give colorless needles; m.p. 178–182° (decomp., inserted at 160°); Beilstein test for halogen negative, Ehrlich test rapidly red cold; R_f "in ammonia" identical with that of its isomer I*h*. Calc. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.50; H, 4.82; N, 6.25.

This preparation could presumably be improved by using the method later developed for I*h*, that is, by washing the crude product with water before extracting into ether.

2-Formylpyrrole-3-propionic acid-4-acetic acid Oxime (IIi)

Hydroxylamine hydrochloride (2 g) was dissolved in 12 ml of water and 8 ml of 10% NaOH added (pH 6–7), then 1 g of the iodopyrrole II*f*. The solution was heated on the steam bath for 1½ hours (gas evolved), cooled to 20°, brought to pH 3 with concentrated hydrochloric acid, and then left overnight at 0°. The precipitate (403 mg) was separated, washed with a little water, then pentane, and dissolved in 4 ml of boiling water. Left at 20° overnight, the solution deposited buff-colored crystals (366 mg, 54%); m.p. 178–180° (decomp., inserted at 160°); Ehrlich test very slowly blue cold; Beilstein halogen test negative. The R_f "in ammonia" (0.33) was exceptional in that it differed from that of the isomeric I*i* (0.29).

For analysis it was recrystallized from water and dried, giving buff-colored crystals, m.p. unchanged. Calc. for $C_{10}H_{12}O_5N_2$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.76; H, 4.89; N, 11.56.

This product appears to remain in supersaturated aqueous solutions more than does its isomer I*i* and to be more strongly retained on Darco. Paper chromatography indicated that it was the sole product when II*c* was treated as in the conversion of I*c* to I*i* by method (*c*), but the isolation of the II*i* resulting by this method has not been worked out.

2-Aminomethylpyrrole-3-propionic acid-4-acetic acid (isoporphobilinogen, IIj)

The oxime II*i* (125 mg) and 250 mg of palladium black were shaken under hydrogen (20°, 1 atm) with 250 ml of redistilled water until absorption ceased after the uptake of 2 moles in 2 hours. The catalyst was filtered off (Whatman No. 1 paper) and the filtrate concentrated to 20 ml in a rotary evaporator (baths at 30° and 0°). The pink solution, after the pH was brought to 7, was filtered through an alumina column (Woelm, neutral, 8 mm diam. × 20 mm, prewashed with water), which was then washed with water until the eluate gave a very faint Ehrlich test in the cold. The eluate (50 ml) was concentrated in a rotary evaporator (baths at 30° and 0°) to about 0.5 ml. After the addition of 0.7 *N* ammonia, the pH was brought to 4–5 with glacial acetic acid (volume of solution now <2 ml). After the solution was scratched, it was left at 0° for 3 hours. The product (83 mg, 70%) was separated and washed with a little water to give colorless needles; m.p. 192–195° (decomp., inserted at 180°); Ehrlich test rapidly red cold; R_f "in acetic acid" identical with that of porphobilinogen. It was also characterized by its X-ray powder photograph and infrared mull spectrum.

For analysis it was recrystallized from 0.7 *N* ammonia – glacial acetic acid and dried (20°, 0.01 mm). Calc. for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.92; H, 6.29; N, 11.96.

Isoporphobilinogen Diethyl Ester Hydrochloride

A solution of 51 mg of isoporphobilinogen, II*j*, in 2 ml of 5% hydrogen chloride in ethanol was left at 0° overnight. The colorless solution was taken to dryness *in vacuo* below 20° and 1 ml of ethanol was three times added and removed as above. The product crystallized on adding ether and seeding, giving 66 mg (92%) of colorless needles; m.p. 97–98.5°; Ehrlich test rapidly red cold; soluble in water and in ethanol. It was also characterized by its X-ray powder photograph and infrared mull spectrum.

For analysis it was recrystallized from ethanol–ether (recovery 88%) and dried (20°, 0.01 mm), m.p. 99–100°. Calc. for $C_{14}H_{23}O_4N_2Cl$: C, 52.74; H, 7.27; N, 8.79; Cl, 11.12. Found: C, 52.68; H, 7.24; N, 8.63; Cl, 11.03.

2-Formyl-5-carboxypyrrole-3-propionic acid-4-acetic acid Triethyl Ester Oxime (Ethyl Ester of IId)

Hydroxylamine hydrochloride (122 mg) in 0.6 ml of water was added to 42 mg of sodium in 10 ml of ethanol and the precipitate was filtered off and washed with ethanol. The filtrate and 500 mg of the ester of IIc (3) were refluxed for ¾ hour. The cooled solution was poured into 175 ml of ice water. After being left overnight at 0°, the product (435 mg, 83%) was filtered off and washed with water, leaving colorless needles, m.p. 104–109°.

For analysis it was twice recrystallized from aqueous ethanol (65% recovery) and dried (57°, 1×10^{-4} mm), m.p. 107–112° softening from 103° (lit. (10), 88°). Calc. for $C_{17}H_{24}O_7N_2$: C, 55.43; H, 6.56; N, 7.61. Found: C, 55.31; H, 6.38; N, 7.79.

2-Aminomethyl-5-carboxypyrrole-3-propionic acid-4-acetic acid Triethyl Ester Hydrochloride (Ester Hydrochloride of IIe)

The ethyl ester of IId (476 mg) was shaken under hydrogen (20°, 1 atm) with 100 mg of palladium black, 50 ml of absolute ethanol, and 0.25 ml of concentrated hydrochloric acid until the uptake of hydrogen ceased (3½ hours, 2 moles taken up). The filtered solution was concentrated to 5 ml *in vacuo*, and 50 ml of ether was added. Scratching induced the separation of the product (386 mg, 76%) as colorless crystals; m.p. 147.5–149°; soluble in 2 *N* HCl; Ehrlich reaction positive hot.

For analysis it was recrystallized from ethanol–ether (recovery 93%) and dried *in vacuo* at 90°, m.p. 147.5–149.5°. Calc. for $C_{17}H_{27}O_6N_2Cl$: C, 52.33; H, 6.96; N, 7.17; Cl, 9.07. Found: C, 52.44; H, 6.93; N, 6.99; Cl, 8.73. The m.p. of the free ester has been reported as 185° (10).

Porphobilinogen Lactam Ethyl Ester (III)

Porphobilinogen lactam (100 mg) (7) was left for 5 hours in 8% ethanolic hydrogen chloride at 20°. The solvent was removed *in vacuo*, then more ethanol was added and removed in the same way. When the residue was crystallized from 3 ml of hot ethanol, two crops of the product were obtained as tan irregular plates (66 mg, 58%), m.p. 239–244° (decomp., inserting at 230°). Calc. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.99; H, 7.23; N, 12.01. The ester was also prepared from the lactam with diazoethane in ether–ethanol.

5-Aminomethyl-5'-carboxypyrromethane-4,3'-diacetic acid-3,4'-dipropionic acid Lactam Tetraethyl Ester (IV)

The ethyl ester of porphobilinogen lactam (III, 47.6 mg) and the ethyl ester of Ib (83.7 mg) (15) were heated for 40 minutes on the steam bath with 1.7 ml of anhydrous sodium acetate in acetic acid (100 mg in 10 ml). Water (4 ml) was added slowly and the mixture heated nearly to a clear solution, then cooled slowly. When a few crystals had separated, the volume was brought to 20 ml with hot water. The nearly colorless amorphous product (81 mg, 70%) was separated from the cooled solution. At about 170° it either melted or changed to crystals, m.p. 181–186°.

For analysis it was extracted by ether (thimble) in which it is very slightly soluble, giving colorless microprisms which at 170° sintered and changed to needles, m.p. 185–189° (hot stage). Calc. for $C_{29}H_{39}N_3O_9$: C, 60.72; H, 6.85; N, 7.33; Found: C, 60.93; H, 6.85; N, 7.21.

The assigned structure is consistent with its U.V. spectrum in ethanol: minimum at 254 mμ and $\epsilon = 15.5\times10^3$ at the 281 mμ maximum. Compare the spectrum of the hexaethyl ester of 5,5'-dicarboxypyrromethane-3,3'-diacetic acid-4,4'-dipropionic acid

($\epsilon = 30.8 \times 10^3$ at 283 mμ (maximum); no minimum) and of the ethyl ester of porphobilinogen lactam (no maximum but a rise toward 210 mμ).

Condensation of 5-Carboxyporphobilinogen with Formic Acid

5-Carboxyporphobilinogen (7) (500 mg), Ie, was heated on the steam bath for ½ hour with 5 ml of 98% formic acid and 2.5 ml of hydrogen bromide (30% in acetic acid). The cooled solution was scratched after the addition of 10 ml of acetic acid then kept at 0° for 1 hour. The crystalline product, formulated as V (472 mg, 72%), was washed with acetic acid, then ether, and twice recrystallized from formic acid – acetic acid (1:4). After being dried overnight at 20° *in vacuo* over sodium hydroxide, the product (57% recovery) formed orange microneedles, decomposing on heating. Calc. for $C_{21}H_{29}O_8N_4Br_3$: C, 35.76; H, 4.14; N, 7.95; Br, 34.00. Found: C, 36.07; H, 3.94; N, 7.49; Br, 33.08.

Adding three equivalents of sodium hydroxide to a solution of the hydrobromide in water precipitated yellow plates, which did not analyze for the free base of the pyrromethene; m.p. about 275° (decomp.); Beilstein halogen test negative. Calc. for $C_{21}H_{26}O_8N_4$: C, 54.54; H, 5.67; N, 12.12. Found: C, 52.08; H, 6.26; N, 14.68.

After the hydrobromide was reduced in dilute sodium hydroxide with sodium amalgam, acetic acid precipitated initially colorless crystals which, after recrystallization, did not analyze well for the pyrromethane. Calc. for $C_{21}H_{28}O_8N_4$: C, 54.30; H, 6.08; N, 12.06. For $C_{21}H_{28}O_8N_4.C_2H_4O_2$: C, 52.67; H, 6.15; N, 10.68. Found: C, 52.84; H, 6.30; N, 10.33.

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PHOTOLYSIS OF POLYACRYLONITRILE[1]

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ABSTRACT

The photolysis of polyacrylonitrile has been investigated in solution (80:20 by weight of ethylene carbonate to propylene carbonate), in powder form, and as a film. Experiments have been carried out in vacuum and in the presence of oxygen. All polymer samples were exposed to ultraviolet light of wavelength 2537 Å. In solution a random chain-scission reaction with a small quantum yield (ca. 10^{-4} main chain bonds broken/quantum absorbed) takes place; at the same time, photochemical reactions occur involving side groups of the polymer chains, as evidenced by the change in the ultraviolet spectra of the polymer solutions. It seems likely that double-bond formation, both conjugated and isolated, and cyclization are responsible for the change in spectra. Infrared spectra do not show any change. Exposed polymer films become cross-linked and exposed powder also cross-links and evolves small molecules, such as nitriles, particularly HCN. Degradation in oxygen suppresses what is believed to be double-bond formation as evidenced by ultraviolet absorption spectra. The photolysis of glutaronitrile as a model substance has also been studied.

INTRODUCTION

Experiments on the photodegradation *in vacuo* of polyacrylonitrile dissolved in a mixture of ethylene carbonate and propylene carbonate (88:20 by weight) and exposed to light of wavelength 2537 Å were presented by Jellinek and Schlueter in a previous paper (1). It was shown that the experimental data could be represented with fair approximation by a random chain-scission process. The small quantum yield which was obtained for the photodegradation is in accord with such a random breakdown process. The rate constants derived on the basis of random degradation were found to be linearly dependent on the incident light intensity.

The present work deals with an extension of the investigation to the photodegradation as a function of polymer concentration, chain length, and history of the polymer samples. In addition, ultraviolet and infrared absorption spectra at various stages of the degradation process have been investigated in detail. The previous results of Jellinek and Schlueter (1) have been re-evaluated by the use of a more appropriate intrinsic viscosity – number-average molecular weight relationship. Further, experiments on the photodegradation of polyacrylonitrile in the form of films and powder and on the production of small molecules during degradation are presented. A model substance, glutaronitrile, has also been investigated.

EXPERIMENTAL

(a) Apparatus

The apparatus was similar to that used previously (1) with only some minor modifications. The irradiations were carried out with a stronger mercury lamp of the same type as employed before. It operated at not more than 600 R.M.S. volts and carried 60 ma instead of 30 ma. Not less than 95% of its light output occurred at 2537 Å. The light source was placed near the reaction vessel, which was located in a thermostat at 25±0.05° C. The light intensities were appreciably greater than those in the previous experiments and a higher experimental accuracy resulted. The lamp was stabilized by a

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constant-voltage transformer. The 30-ma lamp used previously was also employed for rate measurements as a function of light intensity.

Ultraviolet absorption spectra were measured on a Beckman spectrophotometer (Model DU) and on a Bausch and Lomb Spectronic 505. Infrared spectra were obtained with a Beckman IR-5.

All polymer solutions were irradiated in cylindrical Ultrasil quartz cells with plane, parallel, polished windows. The solutions were evacuated to 10^{-5} mm Hg or better and sealed off before exposure.

Intrinsic viscosities of the polymer samples were determined in Ostwald–Fenske viscometers. The measurements were carried out at 25±0.05° C. All intrinsic viscosities are expressed in $(g/100\ ml)^{-1}$.

(b) Materials

Polyacrylonitrile samples of number-average molecular weights 99,100, 54,590, and 26,320 were obtained from the Chemstrand Corporation. Their intrinsic viscosities in dimethylformamide and ethylene carbonate – propylene carbonate (80:20 by weight) respectively were $[\eta]_{DMF}$: 2.19, 1.40, 0.81, and $[\eta]_{EPC}$: 1.65, 1.21, 0.78.

The number-average molecular weights were derived from an equation by Cleland and Stockmayer (2) for dimethylformamide solutions: $[\eta]_{DMF} = 0.392 \times 10^{-3} M_n^{0.75}$.

A polymer sample was also prepared in the authors' laboratory. The procedure was as follows.* Acrylonitrile supplied by Eastman Kodak was washed successively with 3 *N* sulphuric acid, 3 *N* sodium carbonate, and water. It was dried with calcium chloride and distilled twice under atmospheric pressure. Ethylene carbonate was used as a solvent for the polymerization and Eastman Kodak azobisisobutyronitrile served as a catalyst. The solution was 0.058 *M* and 0.0135 *M* with respect to monomer and catalyst respectively. The polymerization was carried out under vacuum at a temperature of 60 to 65° C. After 45 minutes, the reaction mixture was poured into well-stirred methanol; the precipitated polymer was filtered off and washed thoroughly with methanol. Subsequently, it was first refluxed with ether (BDH Analar) for about 1 hour and then refluxed, filtered, and washed with methanol. The refluxing with methanol was repeated to ensure that the polymer was properly purified. The polymer sample was then dried to constant weight in a vacuum oven at 40° C. Its intrinsic viscosity in dimethylformamide and the solvent mixture was respectively 0.50 and 0.54, corresponding to a number-average molecular weight of 13,800.

The solvent mixture for the irradiation experiments was the same as that used previously (1): 80:20 by weight of ethylene carbonate to propanediol cyclic carbonate. The purification methods for these compounds were similar to those employed before.

Ethylene carbonate was accepted to be of sufficient purity when an 80:20 mixture by weight of the carbonate with optically pure methanol gave an optical density (1 cm) of less than 0.06 at 2537 Å, measured against distilled water. The melting point of the carbonate after purification was 34–35° C.

The 1,2 propanediol cyclic carbonate was purified by passage through columns of activated charcoal (BDH Norit) mixed with small amounts of adsorption alumina (Fisher).

The resultant 80:20 mixture by weight of the carbonates was dried in vacuum and had an optical density (1 cm) of 0.05 at 2537 Å.

All other materials used in this work were of Analar grade.

**The procedure was supplied by L. H. Peebles, Jr., of the Chemstrand Corporation.*

(c) Re-evaluation of Previous Experimental Results (1)

In the previous work all number-average chain lengths were evaluated by means of an equation obtained by Onyon (4) for dimethylformamide solutions:

$$[\eta]_{DMF} = 1.97\times10^{-3}\, M_n^{0.625}.$$

By measuring also intrinsic viscosities in the ethylene carbonate – propylene carbonate mixture, the following equation could be derived:

$$[\eta]_{EPC} = 8.9\times10^{-3}\, M_n^{0.47}.$$

All number-average chain lengths of the previous work were recalculated using the Cleland–Stockmayer (2) equation:

$$[\eta]_{DMF} = 0.392\times10^{-3}\, M_n^{0.75}.$$

Here again an equation was derived for the solvent mixture based on the above equation:

[1] $$[\eta]_{EPC} = 2.65\times10^{-3}\, M_n^{0.56}.$$

This equation was used for the recalculation of all the chain lengths. This resulted in different degradation rate constants k_{exp} derived from the equation

[2] $$\frac{1}{P_t} - \frac{1}{P_0} = k_{exp}t,$$

which is valid for a random chain-scission (3) process. P_0 and P_t are the number-average chain lengths at the beginning and at time t of the irradiation process, respectively. All subsequent calculations in this work were carried out using equation [1].

The new rate constants, derived from Jellinek and Schlueter's work, plotted against the relative light intensities give now a straight line passing through the origin of the co-ordinate system, as Fig. 1 shows. Previously, the straight line did not pass through the origin of the co-ordinate system. This could not be explained at the time.

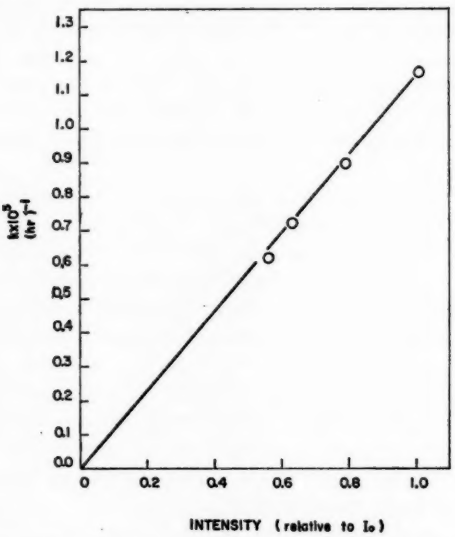


FIG. 1. Experimental rate constants as a function of relative light intensity (re-evaluated data from Jellinek and Schlueter (1)).

(d) Photodegradation as a Function of Polymer Concentration

Figure 2 shows intrinsic viscosities plotted as a function of time of irradiation for all

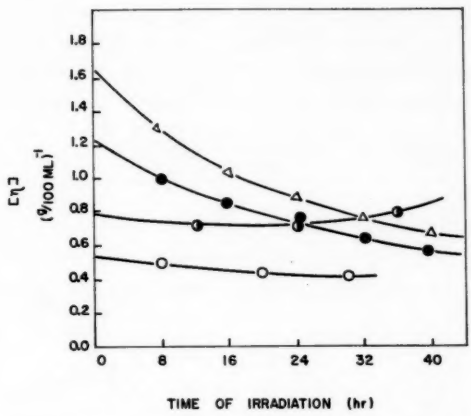


FIG. 2. Intrinsic viscosity as a function of irradiation time. Solutions (0.1% w/v) of PAN in ethylene carbonate – propylene carbonate (80:20 by weight): △ $[\eta]_{EPC,\,0} = 1.65$; ● $[\eta]_{EPC,\,0} = 1.21$; ◑ $[\eta]_{EPC,\,0} = 0.78$; ○ $[\eta]_{EPC,\,0} = 0.54$, homemade sample.

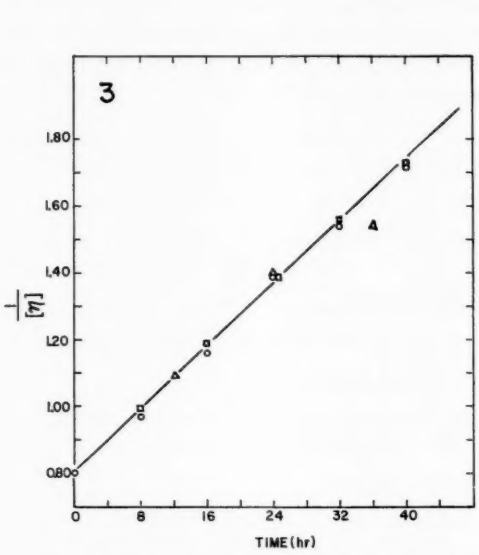
the samples. One sample of intrinsic viscosity $[\eta]_{EPC} = 0.78$ is quite abnormal. Its ultraviolet spectrum also does not agree with those of the other samples. In contrast to the other solutions, solutions of this sample turned distinctly yellow during degradation and showed appreciable fluorescence. This sample apparently cross-links, probably due to impurities. This was not further investigated.

The effect of polymer concentration on degradation was studied with the sample of $[\eta]_{EPC} = 1.21$ over a range of concentrations (0.1%, 0.2%, and 0.4%, w/v). The results are shown in Figs. 3 and 4, where $1/[\eta]_{EPC}$ and $1/P_t - 1/P_0$ respectively are plotted against the time of irradiation. Apparently, the rate is independent of the polymer concentration. The rate constant k_{exp} for equation [2], derived by the least-squares method, is 6.57×10^{-5} hr^{-1}. The number of quanta incident on the reaction vessel for these experiments was 2.58×10^{17} quanta/sec. The reaction vessel had a cross section of 11 cm^2.

(e) Photodegradation as a Function of Initial Polymer Chain Length

Figures 5 and 6 show plots of $1/[\eta]$ and $1/P_t - 1/P_0$ against irradiation time for 0.1% w/v polymer solutions. The rate constants for the two Chemstrand samples, which have similar histories, are quite close, whereas the homemade sample has an appreciably greater rate constant. It is also noteworthy that the $1/[\eta]$ versus t plots are straight lines with identical slopes for all samples. This is not the case when plotting $1/P_t - 1/P_0$ against time. The rate constants are given below.

P_0	$[\eta]_{EPC,\,0}$ ((g/100 ml)$^{-1}$)	$k_{exp} \times 10^5$ (hr^{-1})	$k' \times 10^2$ (100 ml hr^{-1} g^{-1})
1886	1.65	6.05	2.25
1030	1.21	6.57	2.25
252	0.54	9.60	2.25



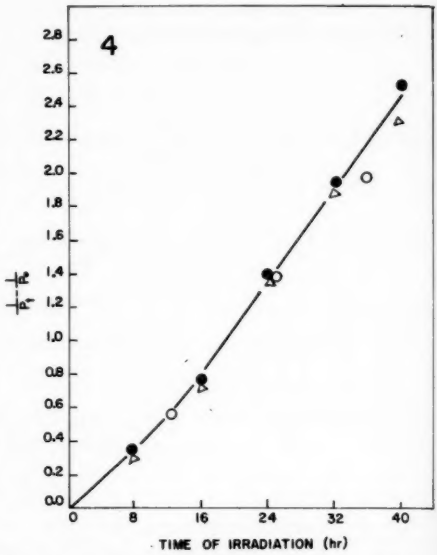
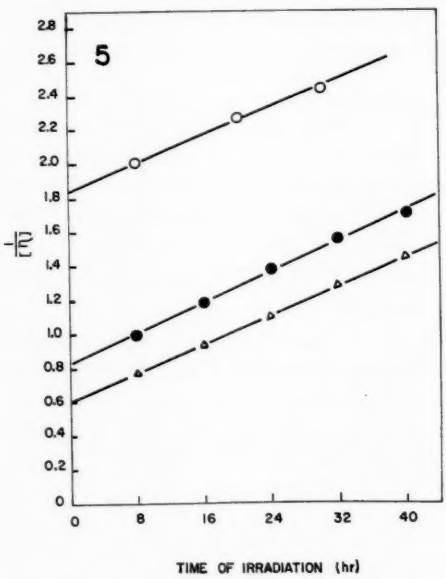


FIG. 3. $1/[\eta]$ as a function of irradiation time for different polymer concentrations (sample of $[\eta]_{EPC,\,0}$ = 1.21): ○ 0.1% w/v; □ 0.2% w/v; △ 0.4% w/v in ethylene carbonate – propylene carbonate.

FIG. 4. $1/P_t - 1/P_0$ as a function of polymer concentration (sample of $[\eta]_{EPC,\,0}$ = 1.21): △ 0.1% w/v; ● 0.2% w/v; ○ 0.4% w/v in ethylene carbonate – propylene carbonate.



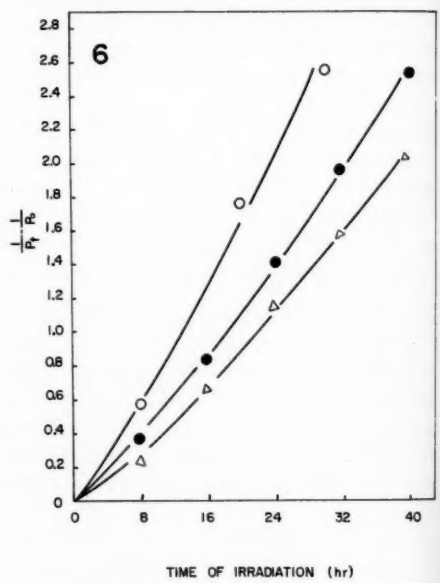


FIG. 5. $1/[\eta]$ as a function of irradiation time for different initial polymer chain lengths (0.1% w/v in ethylene carbonate – propylene carbonate): ○ $[\eta]_{EPC,\,0}$ = 0.54, homemade sample; ● $[\eta]_{EPC,0}$ = 1.21; △ $[\eta]_{EPC,\,0}$ = 1.65.

FIG. 6. $1/P_t - 1/P_0$ as a function of irradiation time for different initial chain lengths (0.1% w/v in ethylene carbonate – propylene carbonate): ○ $[\eta]_{EPC,\,0}$ = 0.54, homemade sample; ● $[\eta]_{EPC,\,0}$ = 1.21; △ $[\eta]_{EPC,\,0}$ = 1.65.

(f) Photodegradation as a Function of Incident Light Intensity

Irradiations were carried out at three incident light intensities on 0.1% w/v solutions of the sample of $[\eta]_{EPC} = 1.21$ (g/100 ml)$^{-1}$. The light intensities were obtained by different positions of the 60-ma lamp and also by employing the 30-ma lamp. The incident intensities were determined in terms of quanta by means of the uranyl actinometer (5). The rate constants obtained at the various intensities were as given below.

Light intensity incident on the 11-cm² reaction cell window $\times 10^{-17}$ (quanta/second)	$k_{exp} \times 10^5$ (hr^{-1})
2.58	6.57
1.67	3.92
0.61	1.52

Figure 7 shows that the rate constants are proportional to the first power of the light intensity.

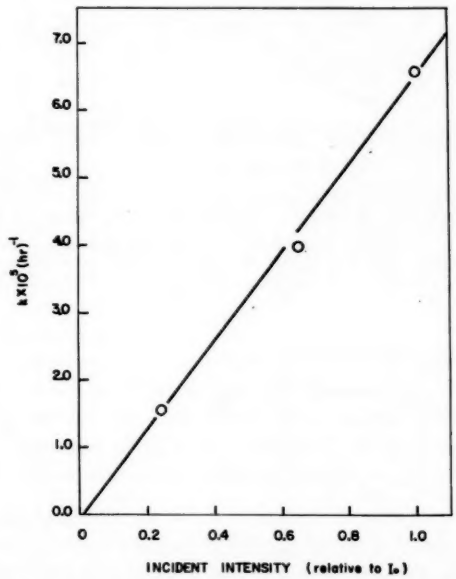


FIG. 7. Experimental rate constants as a function of relative light intensity (0.1% w/v of $[\eta]_{EPC}$, θ = 1.21).

(g) Ultraviolet Absorption Spectra

Figure 8 shows the ultraviolet absorption spectra of the polyacrylonitrile (PAN) sample of $[\eta]_{EPC} = 1.21$ exposed in 0.1% w/v solutions at different stages of the degradation. A pronounced absorption maximum develops at 2950 Å during the irradiation. In Figs. 9 and 10, optical densities at 2537 Å and 2950 Å respectively are given as a function of irradiation time.

Subsequently it was found, using the Bausch and Lomb Spectronic 505, that PAN also has an adsorption maximum at 2160 Å which changes during irradiation. This is shown in Fig. 11.

The homemade sample follows similar changes in its ultraviolet spectra as is the case with the two other samples, as Fig. 12 shows.

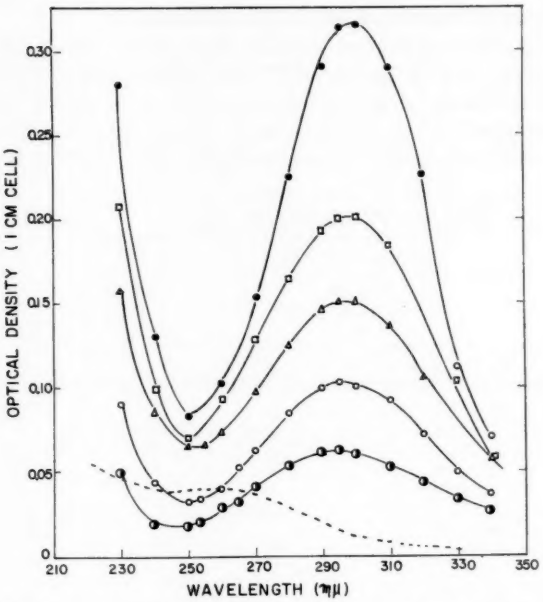


FIG. 8. Ultraviolet absorption spectra of sample of $[\eta]_{EPC,\,0} = 1.21$ as a function of irradiation time 0.1% w/v solutions): – – – unexposed; ◑ 8 hours; ○ 16 hours; △ 24 hours; □ 32 hours; ● 40 hours.

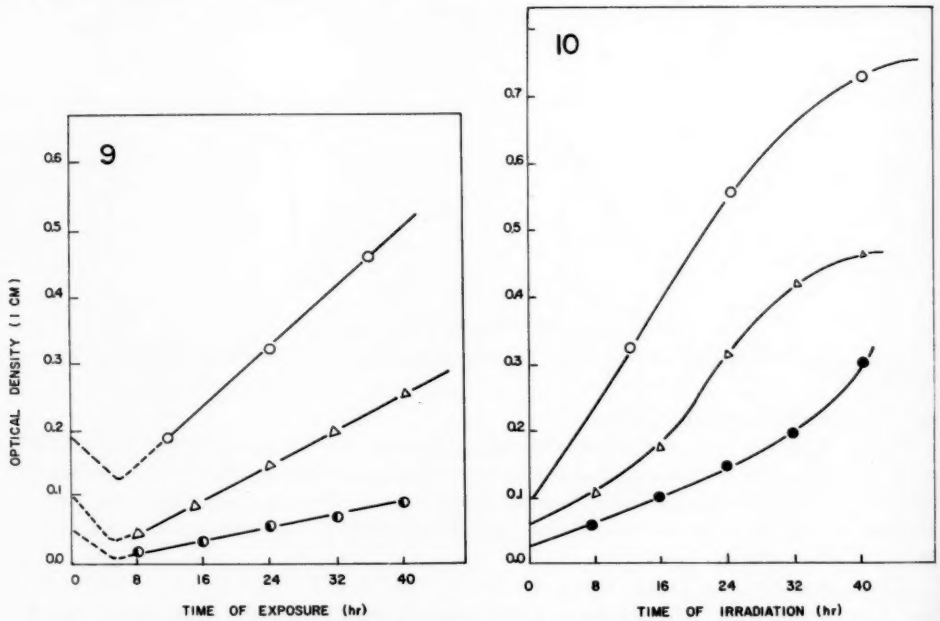


FIG. 9. Optical densities (1 cm) at 2537 Å as a function of irradiation time for sample of $[\eta]_{EPC,\,0} = 1.21$ at different concentrations: ◑ 0.1% w/v; △ 0.2% w/v; ○ 0.4% w/v.

FIG. 10. Optical densities (1 cm) at 2950 Å as a function of irradiation time for sample of $[\eta]_{EPC,\,0} = 1.21$ at different concentrations: ● 0.1% w/v; △ 0.2% w/v; ○ 0.4% w/v.

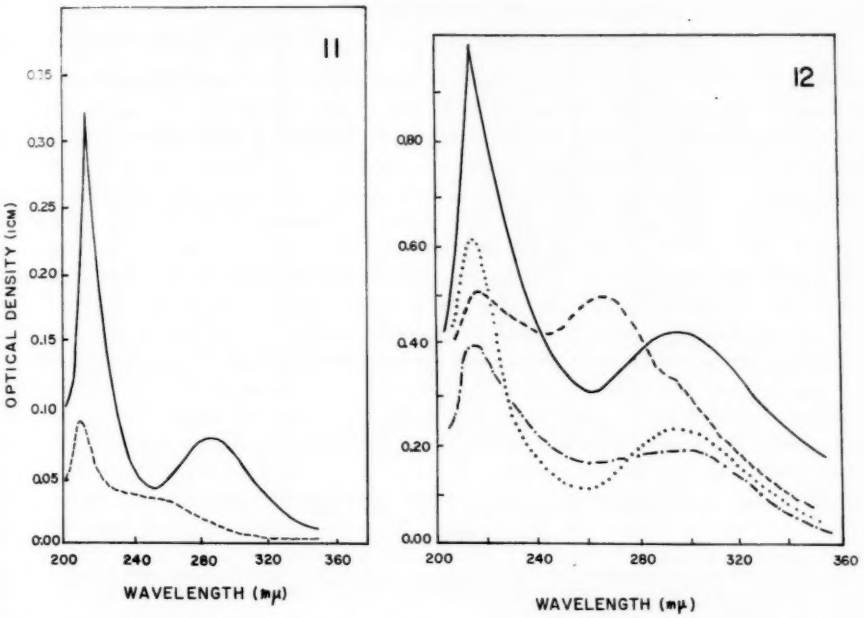


FIG. 11. Optical density (1 cm) of unexposed (– – –) and exposed for 4 hours (——) PAN, $[\eta]_{EPC,\,0} = 1.21$, 0.1% w/v, showing change in absorption at 2160 Å.

FIG. 12. Absorption spectra of 'homemade' PAN, $[\eta]_{EPC,\,0} = 0.54$, 0.1% w/v, as a function of irradiation time: – – – unexposed; –·– 8 hours; . . . 20 hours; —— 30 hours.

(h) Infrared Absorption Spectra

One per cent w/v solutions of PAN of $[\eta]_{EPC} = 1.21$ (g/100 ml)$^{-1}$ were exposed under vacuum for 40 hours. Samples of unexposed and exposed polymer were precipitated from solution by distilled water; the precipitate was washed with water and dried under vacuum. A doublet appeared at 1770 to 1780 cm^{-1}, which could be shown to be due to residual solvent. No other significant changes could be detected.

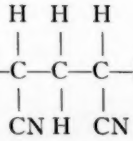
(i) Oxidative Photodegradation

A 1% w/v solution of PAN ($[\eta]_{EPC} = 1.21$ (g/100 ml)$^{-1}$) was irradiated under vacuum for 16 hours. The solution was diluted to 0.5% w/v, and its ultraviolet absorption spectrum determined. The solution was then saturated with oxygen and exposed again. The absorption spectra at various stages of the irradiation process are shown in Fig. 13.

It is apparent that on irradiation in the presence of oxygen, the maximum at 2950 Å is gradually eliminated, indicating that the group responsible for this maximum is subject to photooxidation.

(j) Photolysis of Glutaronitrile as a Model Substance

One per cent w/v solutions of glutaronitrile,



in the solvent mixture were exposed under vacuum to the ultraviolet light source. Ultraviolet absorption

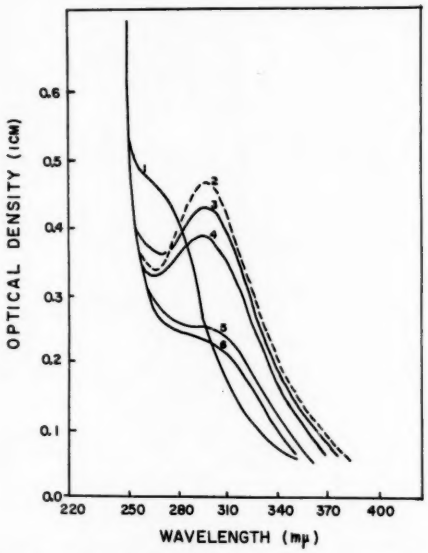


FIG. 13. Oxidative photodegradation of PAN ($[\eta]_{EPC, 0} = 1.21$): (1) 1% w/v, unexposed; (2) 0.5% w/v, 16 hours in vacuum; (3) 0.5% w/v, 10 minutes in O_2; (4) 0.5% w/v, 20 minutes in O_2; (5) 0.5% w/v, 60 minutes in O_2; (6) 0.5% w/v, 120 minutes in O_2.

spectra of the unexposed and exposed solutions are shown in Fig. 14. It is seen that glutaronitrile shows characteristics very similar to those of PAN in the same solvent. The absorption maxima at 2160 Å increased and a new peak at 2950 Å appeared during

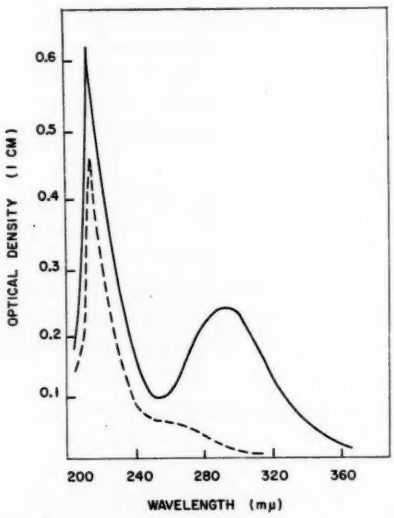


FIG. 14. Photolysis of glutaronitrile, 1% w/v in 80:20 by weight ethylene carbonate – propylene carbonate: – – – unexposed; ——— 12 hours in vacuum (optical density (1 cm) of 0.33% w/v solution).

irradiation. This indicates that changes in the absorption spectra in PAN do not need to be directly related to the chain-scission reaction in the polymer.

(k) Photodegradation of PAN in Bulk and Production of Small Molecules During Irradiation

PAN ($[\eta]_{EPC} = 1.21$ (g/100 ml)$^{-1}$) was degraded in powder form and in the form of films. The powder was irradiated for 4 hours under vacuum; volatile products were collected in a trap cooled by liquid air. On removal of the liquid-air bath and addition of an alcoholic tincture of gum guaiac and 1:1000 $CuSO_4$ solution (6), a dark blue color appeared, indicating the presence of small molecules such as nitriles and ammonia. The product had a distinct smell of HCN. The exposed powder showed a light brown color and was insoluble in dimethylformamide; apparently cross linking had taken place.

PAN films ($[\eta]_{EPC} = 1.21$ (g/100 ml)$^{-1}$) were prepared by evaporating, under vacuum, dilute solutions of PAN in dimethylformamide. The films were dried under vacuum for at least 24 hours. Their thickness was approximately 30 μ. They were exposed to the ultraviolet light source both under vacuum and in the presence of air for about 6 hours. Loss of weight, either by weighing or with a quartz spring, could not be detected. However, for the same time of exposure, the films turned to a yellow color when exposed in air and to a dark brown color in vacuum. The exposed films were insoluble in dimethylformamide. Upon being heated on a steam bath, part of the films dissolved, leaving colored material in suspension.

One per cent w/v solutions exposed under vacuum gave positive tests for small molecules collected in a trap cooled by liquid air.

DISCUSSION

The experimental results agree quite satisfactorily with a random chain-scission process, although the rate constants depend to a certain extent on the history of the polymer. Also reactions other than chain scission take place simultaneously. In a recent note (7), the kinetics for a photodegradation process in solution leading to random chain scission was derived. The theory for films is considered in a different paper (8). It was assumed that the rate of breaking links is directly proportional to the absorbed light intensity, all main chain links being of equal strength. It is further required for a random chain-scission process that an appreciable decrease in the number-average chain length should take place and that the amounts of monomer produced should be negligible (3). Other assumptions made are as follows: (*a*) the absorbed light intensity remains constant during the degradation process, (*b*) Beer–Lambert's law is obeyed and, (*c*) the number of main chain scissions remains small. It was experimentally shown that the polymer solutions obey Beer–Lambert's law; also the number of chain scissions is small in all present experiments. Assumption (*a*) is not fulfilled; the absorption at 2537 Å first decreases and then increases linearly with the extent of degradation. However, this does not necessarily mean that the light absorption with respect to the main chain-scission process does not remain constant. According to the above assumption, the rate of breaking links is given by

$$-\frac{dn}{dt} = k_1 I_{abs}, \qquad [3]$$

where n is the concentration of C—C links in the chain of the polymer (number of links per unit volume) at time t of the irradiation process; k_1, a rate constant; and I_{abs}, the

absorbed light intensity (I_{abs} is the light absorbed per second over the length and 1 cm² of the cross section of the reaction cell). Equation [3] can also be written as

$$-\frac{dn}{dt} = \phi I'_{abs},$$

where ϕ is the quantum yield and I'_{abs} is the average number of quanta absorbed per cm³ per second. Equation [3] leads to (7)

$$[4] \qquad \frac{s}{P_0} = \frac{1}{P_t} - \frac{1}{P_0} = \frac{k_1(1-e^{-k_2 n_0 l})I_0 t}{n_0},$$

where P_t and P_0 are the number-average chain lengths at time t and $t = 0$ respectively; I_0, the incident light intensity for each cm²; n_0, the concentration of main chain links at the beginning of the degradation process (as only a small number of links are broken during the degradation process, n_0 is practically constant); s, the average number of links broken in each original chain at time t; k_2, a constant; and l, the length of the reaction vessel. Further, $k_2 = 2.303E/(n_0 l)$, where $E = \log(I_0/I_t)$ is the optical density of the solution and I_t is the transmitted light intensity. For small light absorption, equation [4] reduces to

$$[5] \qquad \frac{s}{P_0} = \frac{1}{P_t} - \frac{1}{P_0} = k_1 k_2 l I_0 t = \phi k_2 I_0 t = \phi \frac{2.303E}{n_0 l} I_0 t,$$

where $\phi = k_1 l$ is the quantum yield given by

$$\phi = \frac{\text{number of main chain links broken in the system}}{\text{number of quanta absorbed by the system}}.$$

Equation [5] shows that for small light absorption, s/P_0 should be independent of initial chain length and polymer concentration and directly proportional to the incident light intensity. Hence $1/P_t$ plotted against time of irradiation should give straight lines, which is approximately the case (see Figs. 4 and 6). The rate constants $k_{exp} = k_1 k_2 I_0$ should be independent of polymer concentration and initial chain length. This is true as far as the dependence on concentration is concerned (see Fig. 4); however, k_{exp} with respect to the initial polymer chain length seems also to be dependent on the history of the polymer. The condition that k_{exp} is directly proportional to the light intensity is very well fulfilled (see Fig. 7).

In the previous work (1), the quantum yield was found to be $\phi = 7.7 \times 10^{-4}$ main chain bonds broken for each quantum absorbed. In the present work, the quantum yield was calculated from equation [5] for one of the experiments (0.1% w/v of polymer of $[\eta]_{EPC} = 1.21$, $k_{exp} = 6.57 \times 10^{-5}$ hr^{-1}). The optical density was averaged for a 40-hour period of irradiation, assuming a straight-line relationship between the optical density at 2537 Å and the time of irradiation. The quantum yield in this case is 0.84×10^{-4} main chain bonds broken per quantum absorbed. This value is of the same magnitude as that obtained previously and is in accord with a random chain-scission process. Quantum yields for other polymers (9) are of similar magnitude except for polymethylmethacrylate which is known to follow a depolymerization process (10).

The small curvature in the $1/P_t$ versus time curves may be due to a number of reasons:

(*a*) The intrinsic viscosity – number-average molecular weight relationship may not

be quite correct; an increase in the exponent of M_n in the relationship would straighten the curves. This is indicated by the fact that straight lines are obtained when $1/[\eta]_{EPC}$ is plotted against the time of irradiation (see Figs. 3 and 5).

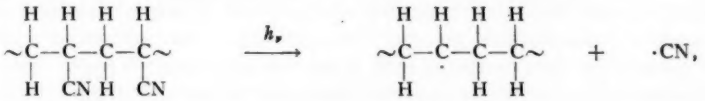
(*b*) The molecular-size distributions change during degradation, which may affect the parameters in the intrinsic viscosity – number-average molecular weight relationship.

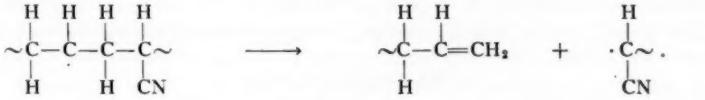
(*c*) The absorbed light intensity does not remain constant during irradiation.

It seems unlikely that the change in absorbed light intensity is the cause of the curvature in the $1/P_t$ versus time curves. Especially during the first 8 hours, a very pronounced change in absorption takes place at 2537 Å, which is not reflected in these curves (see Figs. 4 and 6). It is likely that the change in absorption spectra has nothing or very little to do with the chain-scission reaction. This is supported by the fact that the absorption spectrum for glutaronitrile changes in a way similar to that of the polymer during irradiation. Thus it is probable that this change in the spectra is mainly due to reactions at the nitrile groups leading to formation of other groups, which then cause the change in the absorption spectra. It appears, therefore, that the light absorption related to the chain-scission process remains reasonably constant, while absorption connected with other photochemical reactions changes appreciably. The most likely explanation for the curvature in the $1/P_t$ versus t curves seems, therefore, to be that the intrinsic viscosity – number-average molecular weight relationship is not quite appropriate for the samples employed in this work.

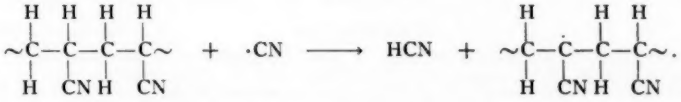
Tentative suggestions as to the reactions involved in the chain-scission process were presented in the previous paper. These reactions can now be elaborated in more detail on the basis of the additional information gained in this work. However, it must be kept in mind that any of the proposed reactions are speculative.

Chain scission may be brought about by the following reactions:

[1] 

[2] 

The ·CN radical could react with an α-hydrogen atom,

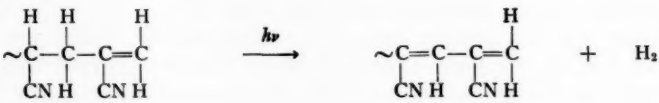
[3] 

Reaction [3] may be followed by another chain scission,

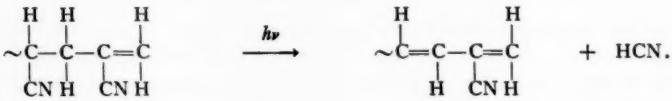
[4] 

The terminal double bond may give rise to the increase of the absorption maximum at 2160 Å.

Two other reactions may take place, not leading to chain scission but producing small molecules:

[5] 

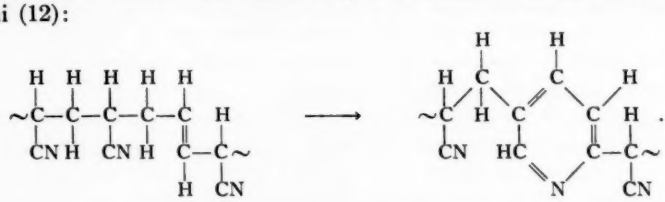
and

[6] 

Such conjugated systems may give rise to the absorption at 2950 Å.

A reaction in which HCN was produced was postulated by Straus and Madorsky (11) for the thermal degradation.

After removal of HCN, cyclization may also occur. This has been formulated by Kobayashi (12):

[7] 

Such substituted pyridines absorb in the 3000-Å region.

In summary, it may be stated that photodegradation of polyacrylonitrile in solution follows a random chain-scission process, although other photochemical reactions also occur at the same time. As a matter of fact, these side reactions are really more extensive than the chain-scission reaction from a chemical point of view, but the scission reaction is more prominent as it affects the molecular weight of the polymer so drastically. Exposure in bulk leads to cross linking and production of small molecules such as HCN.

ACKNOWLEDGMENTS

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STEREOCHEMICAL STUDIES

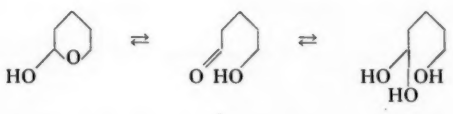
III. REACTIONS OF 4-OXA-5α-CHOLESTAN-3α-OL, A CARBOHYDRATE MODEL[1]

J. T. EDWARD, P. F. MORAND,[2] AND I. PUSKAS[2]

ABSTRACT

4-Oxa-5α-cholestan-3α-ol was prepared by reduction of 4-oxa-5α-cholestan-3-one with lithium aluminum hydride. It showed several typical carbohydrate reactions, such as mutarotation in aqueous tetrahydrofuran and condensation with alcohols in the presence of hydrogen chloride to give 3-alkoxy-4-oxa-5α-cholestanes. These compounds were also prepared by the reaction of alcohols with 3α-chloro-4-oxa-5α-cholestane in the presence of base. Factors governing the proportions of 3α and 3β isomers formed in these reactions are discussed.

The study of the pyranose ring system in simple compounds (e.g. I), devoid of the many hydroxyl groups and asymmetric carbon atoms of the common sugars, has attracted some interest (1, 2, 3, 4). Hurd and Saunders (5) showed that tetrahydropyran-2-ol (I) (6) in 75% aqueous dioxan had an ultraviolet absorption peak at 287 mμ, indicating the presence of the open-chain tautomer, 5-hydroxypentanal (II). From a comparison of the intensity of this peak with that of 5-methoxypentanal, they estimated that 5-hydroxypentanal made up 6% of the equilibrium mixture. They assumed the remaining 94% to be entirely in the lactol form I, but it is likely that about 2–3% is present as the hydrated aldehyde III (7, 8).



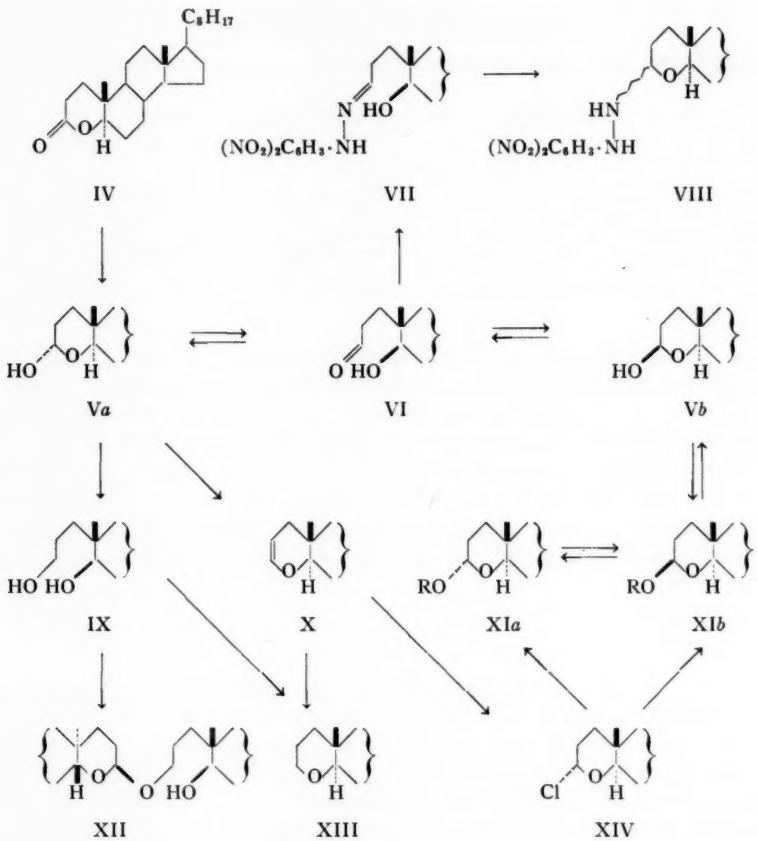
I II III

Substitution of the lactol stabilizes it with respect to the open-chain tautomer, which at equilibrium is present to only a very minor extent in solutions of the common pentoses and hexoses (9), perhaps because of conformational effects (10).

There appear to be advantages in studying the reactions of the lactol fused to a rigid polycyclic system, as in V. The use of optically active compounds makes it possible to determine the stereochemical course of reactions from the optical rotations of products, while the interpretation of results is simplified by the fact that the pyranose ring is locked into the C-1 conformation, the more stable conformation of glucose and many other pyranoses (11).

To obtain the desired lactol (V), 4-oxa-5α-cholestan-3-one (IV) (12, 13) in tetrahydrofuran was reduced with one-quarter of a mole of lithium aluminum hydride (14). A crystalline product, $C_{26}H_{46}O_2$, was obtained which possessed a potential carbonyl group, as shown by its reaction with 2,4-dinitrophenylhydrazine to form a derivative, $C_{32}H_{50}O_5N_4$. The infrared spectrum of this derivative had no hydroxyl peak, indicating that it had the cyclic structure VIII rather than the structure VII. Such cyclic structures have been postulated for the phenylhydrazones of some pyranoses, although the evidence for them is equivocal (15).

[1]*Manuscript received June 5, 1961.*
Contribution from the Department of Chemistry, McGill University, Montreal, Que.
[2]*Holders of N.R.C. Studentships, 1957–59 (P. F. M.), and 1959–61 (I. P.).*



The reduction product itself is also in a cyclic form (V), since no carbonyl peak characteristic of the hydroxyaldehyde VI could be detected in the ultraviolet absorption spectrum of the compound in 90% aqueous dioxan or 85–90% aqueous tetrahydrofuran, or in the infrared absorption spectrum of the compound as a solid or in solution in chloroform, carbon tetrachloride, or tetrahydrofuran. Evidently fusing the lactol I to the rigid BCD ring system of cholesterol stabilizes the cyclic form.

The α-configuration (V*a*) of the 3-hydroxyl group of the crystalline lactone V is shown by the molecular rotation of the lactol, given in Table I along with the values calculated for the 3α (V*a*) and 3β (V*b*) compounds (using the known value for the parent compound (XIII)) by the elegant and powerful method of Brewster (16). Calculation by Whiffen's method (17) gives almost the same values; however, Whiffen's parameters are considered to be slightly less reliable in the present application, since for the most part they are derived from rotations of compounds dissolved in water rather than in organic solvents. The effect of solvent on rotation, not taken in account in Whiffen's or Brewster's treatments, is appreciable for our compounds (cf. Table I), but not so large as to invalidate the use of these treatments for assigning configurations.

TABLE I

Molecular rotation $[M]_D$ values of 3-substituted 4-oxa-5α-cholestanes

3-Substituent	Compound	$[M]_{D,\ calc}$ (deg)	$[M]_{D,\ obs}$ (deg)	Solvent*
(Hydrogen)	XIII	(+186)	+180†	$CHCl_3$
			+189	CCl_4
			+190	THF
α-Hydroxy	V*a*	+286	+250	$CHCl_3$
			+256	CCl_4
			+344	THF
			+356	THF–H_2O (9:1, v/v)
β-Hydroxy	V*b*	+186	—	
α-Methoxy	XI*a*, R = Me	+391	+442	$CHCl_3$
			+410	CCl_4
			+460	THF–MeOH (1:1, v/v)
β-Methoxy	XI*b*, R = Me	+81	—	
α-Benzyloxy	XI*a*, R = CH_2Ph	+477‡	+466	$CHCl_3$
			+436	THF–$PhCH_2OH$ (2:3, v/v)
β-Benzyloxy	XI*b*, R = CH_2Ph	−5‡	−115	$CHCl_3$
			−19.2	THF–$PhCH_2OH$ (2:3, v/v)
α-Chloro	XIV	(ca. +686)	+672	$CHCl_3$
			+666	CCl_4
			+666	THF
β-Chloro		+186	—	

*THF = tetrahydrofuran.
†Fieser *et al.* (18) report the same value.
‡Calculated from the average increment in molecular rotation (+191° and −191°) in forming α- and β-benzyl-D-glycosides from the respective anomers (19).

While the optical rotation of the lactol V*a* dissolved in chloroform, carbon tetrachloride, or tetrahydrofuran showed no change on standing, it dropped rapidly for solutions in 85–90% aqueous tetrahydrofuran (Fig. 1), and with 1% hydrochloric acid

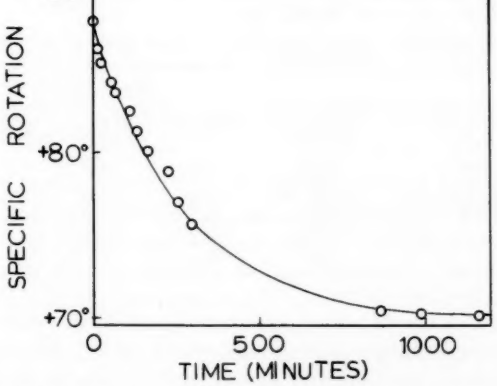


FIG. 1. Change in specific rotation of 4-oxa-5α-cholestan-3α-ol in 90% aqueous tetrahydrofuran (v/v) at 21° C. Theoretical curve for a first-order reaction having a rate constant of 0.0038 min^{-1} (natural logarithms).

in this solvent it had dropped to a steady value in less than 5 minutes. This change in rotation indicated the formation of the β isomer V*b*; such mutarotation is known to be catalyzed by acid (20). Assuming for the 3β isomer V*b* the molecular rotation shown in Table I, the lactol in 90% aqueous tetrahydrofuran is made up of 55% of the α isomer and 45% of the β isomer. However, all attempts to isolate the β isomer by crystallization from different solvents failed, only the α isomer being obtained.

Treatment of the lactol V*a* with hot acetic anhydride – sodium acetate gave 4-oxa-5α-cholest-2-ene (X), more conveniently prepared by the action of phosphorus oxychloride in pyridine. The acetate (XI, R = CH_3CO) could be prepared by the action of acetic anhydride in pyridine at 20° C, but proved reactive and difficult to purify; attempts to crystallize it from methanol afforded 3α-methoxy-4-oxa-5α-cholestane (XI*a*, R = Me), described below. Hydrogenation of the unsaturated ether X gave 4-oxa-5α-cholestane (XIII), previously obtained by dehydration of the diol IX (13).

Like dihydropyran (21), 4-oxa-5α-cholest-2-ene (X) in ether added hydrogen chloride to give a reactive chloro ether, which in the present instance must be 3α-chloro-4-oxa-5α-cholestane (XIV). This addition reaction may be reversible, like that of dihydropyran (21), because infrared studies showed the chloro ether always to be contaminated with small amounts of the unsaturated ether X, and attempts to purify it by crystallization from inert solvents always led to a drop in melting point. However, when a solution of the chloro ether in carbon tetrachloride ($[\alpha]_D$ +153°), shown by quantitative infrared measurements to contain 4% of the unsaturated ether, was saturated with hydrogen chloride, the specific rotation rose to +163° and the infrared peaks due to the unsaturated ether disappeared. The molecular rotations recorded in Table I for this compound are all for solutions saturated with hydrogen chloride.

The α-configuration at the 3-position of the chloro ether is probable from its molecular rotation (Table I). The molecular rotation of 3β-chloro-4-oxa-5α-cholestane should, according to the treatments of Whiffen and Brewster, be about the same as that of the parent compound (XIII); the molecular rotation of the 3α-chloro compound should be different, but the parameters which enable one to calculate it are not available. However, it is evident from Table II that the introduction of an α-chlorine atom at the 1-position

TABLE II

Molecular rotation values $[M]_D$ (deg) of sugar derivatives

Parent compound	Derivatives				
	1-Deoxy	1α-Chloro	$\Delta[M]_D$	1β-Chloro	$\Delta[M]_D$
3,4,6-Tri-*O*-acetyl-D-glucopyranose	+129 (22)	+608 (23)	+479	−48 (24)	−177
2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose	+25 (25)	+600 (26)	+575	+62 (27)	+ 37
2,3,4,6-Tetra-*O*-acetyl-D-mannopyranose	−140 (28)	+331 (29)	+471		

of a pyranose ring in the C-1 conformation (11) causes a shift in molecular rotation of about +500°, the nature and configuration of the adjoining asymmetric center having a comparatively minor effect, so that the molecular rotation of 3α-chloro-4-oxa-5α-cholestane (XIV) should be about +686°, in good agreement with the values found for our chloro ether.

This argument by itself cannot be considered completely conclusive because the

evidence for the configurations assigned to the glycosyl halides is generally indirect and dependent on an interpretation of the stereochemical course of reactions (30). However, the configurations assigned to the stable glycosyl halides (30) and to 3α-chloro-4-oxa-5α-cholestane are those to be expected from the "anomeric effect" (31, 32, 33), discussed further below, which predicts that in dihydropyran rings a halogen atom on the carbon atom adjacent to the ring oxygen will be more stable in the axial orientation.

The lactol V*a* in methanol containing 3% hydrogen chloride gave an 88% yield of a crystalline methoxy derivative, shown by its rotation (Table I) to be 3α-methoxy-4-oxa-5α-cholestane (XI*a*, R = Me). This compound was stable in alkaline solution, but in aqueous acid was readily hydrolyzed to the lactol. Second crops from the preparation of the ether (XI*a*, R = Me), when obtained by rapid evaporation of the solvent at a low temperature (i.e. under non-equilibrating conditions (see below)), had a shoulder at 1068 cm^{-1}, absent in the infrared spectrum of the pure 3α isomer, which is probably due to the 3β isomer (XI*b*, R = Me). However, the latter could not be isolated in a pure state.

A solution of 3α-methoxy-4-oxa-5α-cholestane in methanol–tetrahydrofuran (1:1, v/v) having a low concentration (0.018 *M*) of hydrogen chloride showed a rapid drop in rotation (Fig. 2); with a 0.06 *M* concentration of hydrogen chloride the rotational drop

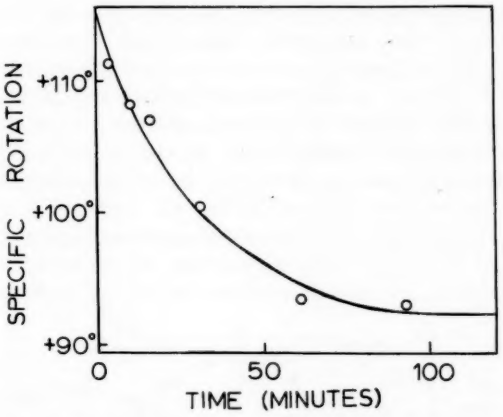
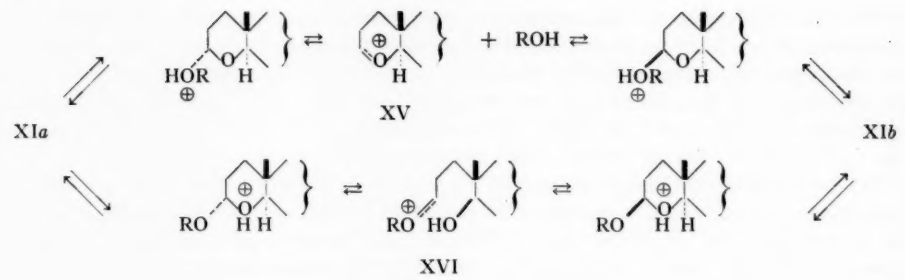


FIG. 2. Change in specific rotation of 3α-methoxy-4-oxa-5α-cholestane in tetrahydrofuran–methanol (1:1, v/v), 0.018 *M* with respect to hydrochloric acid, at 23° C. Theoretical curve for a first-order reaction having a rate constant of 0.040 min^{-1}.

was too fast to be measured. The solid recovered from the neutralized solution after equilibrium had been reached showed again a shoulder at 1068 cm^{-1}. These changes can be due only to the partial isomerization of the 3α (XI*a*, R = Me) to 3β (XI*b*, R = Me) compound. In methanol containing an acid these compounds would be expected to be in equilibrium via the intermediate cyclic (XV) (31) or acyclic carboxonium ion (XVI) (34).

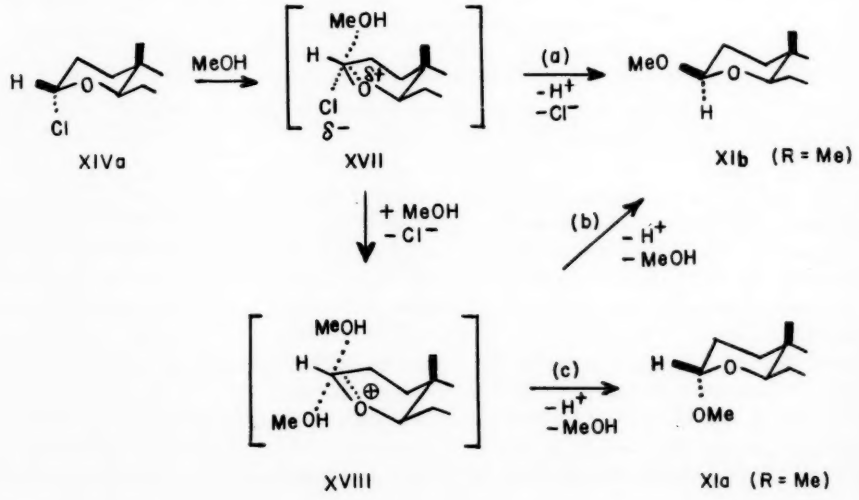
Accepting the calculated value (Table I) for the optical rotation of the 3β isomer, the change in rotation indicates a 24% conversion of the 3α to the 3β isomer.

The rate of this isomerization is considerably faster than that of α-methyl-2-deoxy-D-galactopyranoside (35), which in turn is faster than that of α-methyl-D-galactopyranoside (36). A progressive decrease in rate with the introduction of electron-attracting



groups is expected because of their effect in decreasing the basicity of the acetal oxygen, and hence the concentration of protonated intermediate, and also because of their effect in decreasing the stability, and hence probably the rate of formation, of the intermediate carboxonium ion (37, 38).

In an attempt to obtain a mixture rich in 3β-methoxy-4-oxa-5α-cholestane (XI*b*, R = Me), from which it might be isolated, the chloro ether XIV was solvolyzed in methanol containing methoxide ion or pyridine to neutralize the acid formed. With pyranosyl chlorides derived from methylated or acetylated sugars, such reactions take place with predominant inversion (30, 39, 40), provided that neighboring groups do not participate (41), and provided that steric hindrance to the entering group is not excessive (40). However, in the present instance the 3α-methoxy compound was the chief product, and no 3β-methoxy compound could be isolated, although its presence was indicated by a strong peak at 1068 cm^{-1}. The displacement of chlorine consequently takes place mainly with retention of configuration at the 3-position. The solvolysis of such a chloride in methanol is known to take place by an S_N1 mechanism (39, 40, 42, 43) and probably gives the solvated ion pair XVII (44). This ion pair would be more stable than the ion pairs formed from the chlorides of pyranose sugar derivatives because of the inductive effects of the many oxygen functions of the latter. Consequently, it would be relatively long lived, and not constrained to give an inverted product (XI*b*,



R = Me) by reacting with methanol before the chloride ion had diffused away (route (*a*)). The reaction of the symmetrically solvated carboxonium ion XVIII might seem equally likely to give the 3β (route (*b*)) and 3α product (route (*c*)); the preference for the latter probably arises from the steric effect of the angular methyl group at the 10-position, which generally favors the formation of α-substituted products in rings A and B of steroids (45). Very recently, Rhind-Tutt and Vernon (40) have observed a similar effect in the solvolysis of 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranosyl chloride in methanol, which proceeded with 41–43% retention of configuration, while the corresponding glucose derivative gave almost complete inversion. This was attributed to steric hindrance in the mannopyranosyl chloride by the axial methoxyl group on the carbon adjacent to the reacting center, a far more severe hindrance than that offered by the angular methyl group of the chloro ether XIV. The fact that, even so, 57–59% inversion occurs in solvolysis of the mannopyranosyl chloride may be explained by the inductive effect mentioned above.*

Reaction of 3α-chloro-4-oxa-5α-cholestane with methanol in the presence of silver carbonate again gave mainly the 3α-methoxy compound, although under these conditions glycosyl halides generally give β-methoxy derivatives (46).

The reaction of 4-oxa-5α-cholestan-3α-ol with benzyl alcohol containing 3% hydrogen chloride gave a syrup from which was obtained, by chromatography on basic alumina (activity grade III), two compounds having the analysis and ultraviolet absorption spectra expected for the benzyl ethers (XI*a* and XI*b*, R = CH_2Ph). Their optical rotations (Table I) indicated one to be the 3α isomer (XI*a*, R = CH_2Ph) and the second to be the 3β isomer (XI*b*, R = CH_2Ph).

In a 0.005 *M* solution of hydrogen chloride in tetrahydrofuran – benzyl alcohol (2:3, v/v) both the 3α ($[\alpha]_D$ +91°) and 3β ($[\alpha]_D$ −4°) isomer underwent mutarotation to give a solution having $[\alpha]_D$ +66° (Fig. 3), and hence containing about 73% of the α and

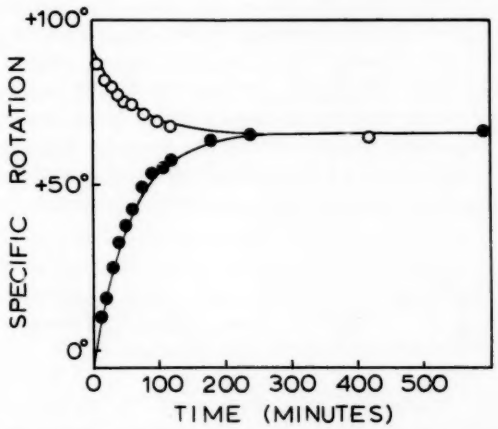


FIG. 3. Change in specific rotation of 3α- (○) and 3β-benzyloxy-4-oxa-5α-cholestane (●) in a 0.0032 *M* solution of hydrogen chloride in absolute tetrahydrofuran – benzyl alcohol (2:3, v/v) at 22.5° ± 0.5°C. Theoretical curves for first-order reactions having a rate constant of 0.0018 min^{-1}.

**Rhind-Tutt and Vernon (40) have claimed that the comparative unreactivity of pyranosyl chlorides derived from hexoses, as compared with simple α-chloro ethers, argues against a half-chair form of the carboxonium ion (31) being generated in solvolysis, with the positive charge localized chiefly on the oxygen. Such an argument ignores the inductive effects operating in derivatives of sugars.*

27% of the β isomer. This interpretation of the rotational changes was confirmed by starting with the pure 3α ether and isolating, after equilibration, 68% of the α and 12% of the β isomer. An attempt was made to catalyze this isomerization also by titanium tetrachloride in carbon tetrachloride, under conditions found successful for some carbohydrate derivatives (29). However, the only crystalline product to be isolated was 4-oxa-5α-cholestan-3α-ol (V*a*), probably formed from 3α-chloro-4-oxa-5α-cholestane when the mixture was treated with water at the end of the reaction.

Reaction of 3α-chloro-4-oxa-5α-cholestane (XIV) with benzyl alcohol in the presence of sodium acetate afforded the 3α and 3β benzyl ethers, isolated in 68% and 9% yields respectively. This is a further example of the solvolysis of the chloro ether proceeding mainly with retention of configuration at the 3-position.

While the benzyl ethers (XI*a* and XI*b*, R = CH_2Ph) would be expected to be fairly stable; in fact, on neutral and basic alumina of activity grade I, decomposition to 4-oxa-5α-cholest-2-ene (X) and 4-oxa-5α-cholestan-3α-ol (V*a*) took place. Consequently, all chromatographic separations were carried out with basic alumina, activity grade III, and even with this adsorbent there was evidence of slight decomposition.

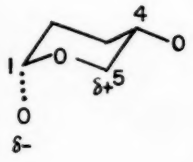
In Table III are summarized the results of the equilibrations of the 3-substituted

TABLE III

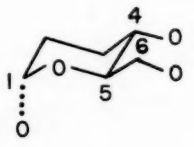
Thermodynamic constants for equilibrations of 3-substituted 4-oxa-5α-cholestanes at 22°±1° C

3-Substituent	Solvent	K	$-\Delta F_{obs}$ (cal/mole)	$-\Delta F_{calc}$ (cal/mole)
OH	Tetrahydrofuran–water (9:1, v/v)	1.2	110	170
OMe	Tetrahydrofuran–methanol (1:1, v/v)	3.1	670	710
OCH_2Ph	Tetrahydrofuran – benzyl alcohol (3:2, v/v)	2.7	590	710

4-oxa-5α-cholestanes expressed as equilibrium constants K (concentration of 3α isomer/concentration of 3β isomer). It is evident that in all cases the axial α isomer is more stable than the equatorial β isomer. This reversal of the usual order of stabilities found in cyclohexane derivatives is undoubtedly due to the ring oxygen, and has been variously explained (31, 32, 33). The most convincing explanation is due to Lemieux and Chu (32, 33), who showed that a consideration of bond lengths, bond angles, and dipoles in the structures XIX and XX indicated an attraction between C-5 and the anomeric oxygen (termed the "anomeric effect") large enough to overcome the steric repulsions from axial hydrogen atoms and hence to favor an axial orientation for the anomeric oxygen atom.



XIX



XX

Also shown in Table III are the free energy differences (ΔF_{obs}) between the 3α and 3β isomers given by the relation $\Delta F = -RT \ln K$. With some isomerizations and conformational changes such free energy differences have been successfully calculated by

additions of terms due to differences in non-bonded interactions in the two isomers (32, 33, 47) or conformers (48). In fact, the repulsion energies giving rise to these non-bonded interaction terms should affect only the enthalpy and not the entropy component of the free energy (49). However, Lemieux and Chu (32, 33) give reasons for believing that the entropy difference is negligible for the anomers of aldopentopyranose tetra-acetates and aldohexopyranose pentaacetates, and this may be true also for the isomeric forms of the ethers (XI, R = Me and CH_2Ph). It is less obviously true for the isomeric forms of the lactol V, as shown by the work of Kabayama, Patterson, and Piche (50) on the entropy differences between α and β forms of pyranose sugars in aqueous solution; however, their interpretation of these differences stresses the importance of the quasi-crystalline structure of water, and it is possible that the differences become less in organic solvents.

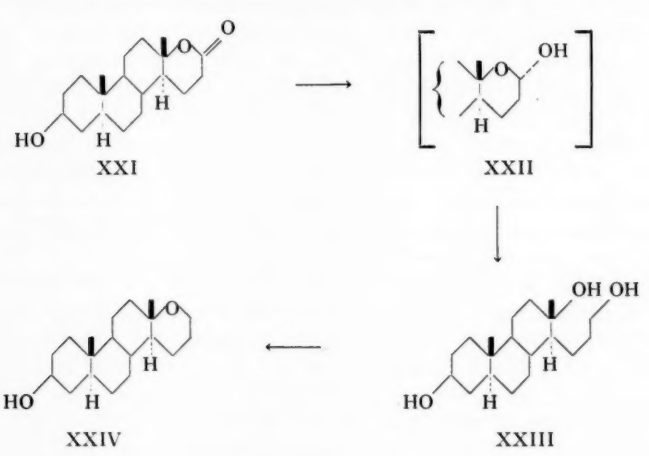
Lemieux and Chu (32, 33) found the free energies of sugar acetates to be additive functions of the following terms (among others): steric repulsion between axial hydrogen and axial oxygen, 180 cal/mole; anomeric effect for pentopyranose derivatives (XIX), 1290 cal/mole; anomeric effect for hexopyranose derivatives (XX), 1510 cal/mole. The first value seemed to be reasonably independent of the nature of the non-aqueous solvent (33), and is adopted here for the methyl and benzyl ethers (XI, R = Me and CH_2Ph), together with a value of 1070 cal/mole for the anomeric effect in these compounds. This latter value is derived on the assumption that the effect is due to the positive charge at C-5 caused by the ring oxygen atom. Addition of another oxygen atom at C-4 should increase the positive charge in the pentose derivatives XIX and give an anomeric effect of $1070+220 = 1290$ cal/mole, while addition of two oxygen atoms, at C-4 and C-6, should give an anomeric effect of $1070+2\times220 = 1510$ cal/mole in the hexose derivatives XX, on the assumption that the inductive effects of the C-4 and C-6 oxygens are the same, and that those of the C-2 and C-3 oxygens may be ignored. The free energy differences between α and β ethers (XI, R = Me and CH_2Ph) calculated using these terms are in reasonable agreement with the values found (Table III).

Chiurdoglu and Masschelein (51) have recently shown that the free energy of the axial hydroxyl – axial hydrogen interaction in cyclohexanol is 150–180 cal/mole, about the same as Lemieux and Chu's value for the axial acetoxyl – axial hydrogen interaction, and independent of the nature of the solvent. However, the most powerful hydrogen-donor solvent investigated by Chiurdoglu and Masschelein was chloroform. There is some evidence that the free energy of the axial hydroxyl – axial hydrogen interaction becomes considerably greater in aqueous or alcoholic solvents (33, 47), and Angyal and McHugh (48) calculated for it a value of 450 cal/mole from studies of the equilibria between cyclitols and their borate complexes in aqueous solution. The free energy difference between α and β forms of the lactol V calculated with this value and a value of 1070 cal/mole for the anomeric effect is again in reasonable agreement with the observed value (Table III), although this agreement may be fortuitous because of neglect of the entropy change.

While the reduction of the lactone IV with 0.25 mole of lithium aluminum hydride gave the lactol V*a*, with 1.2 mole of hydride it gave the diol IX (13), also obtained from the lactol V*a* by hydride reduction. In one experiment in which the lactone was reduced with 0.4 mole of hydride, a syrupy product was isolated, which, by chromatography on alumina, yielded 4-oxa-5α-cholest-2-ene, 4-oxa-5α-cholestan-3α-ol, a solid (product A), and 3,5-seco-A-norcholestan-3,5β-diol (IX). Product A was indicated by analysis to be composed of one molecule of 4-oxa-5α-cholestan-3α-ol and two molecules

of 3,5-seco-A-norcholestan-3,5β-diol, less one molecule of water; in agreement with this formulation, it gave, on treatment with methanolic hydrogen chloride, 3α-methoxy-4-oxa-5α-cholestane and the diol in about the expected proportions. It seems likely that it is a stable complex of one molecule of the structure XII with one molecule of diol, possibly formed when the eluent from the chromatogram containing the lactol V and diol was concentrated. The molecular rotation (+654°) supports this structure, being close to the value (+641°) calculated from the sum of the rotations of two molecules of diol and one molecule of 3α-alkoxy-4-oxa-5α-cholestane (assumed to have the same molecular rotation as the 3α-methoxy derivative). In camphor the complex is dissociated, a Rast determination of molecular weight indicating about half the theoretical value.

Attempts were made to prepare the lactol XXII, in which the steric repulsion of the angular methyl group would be expected to outweigh the anomeric effect, and which in consequence should have an equatorial hydroxyl group. However, reduction of the lactone XXI (52) with varying quantities of lithium aluminum hydride gave only starting material or the completely reduced diol XXIII. The latter was cyclized to 17α-oxa-D-homo-5α-androstan-3β-ol (XXIV) with benzenesulphonyl chloride in lutidine (cf. ref. 13).



EXPERIMENTAL

Where concentrations are not given, specific rotations are averages of 2–3 determinations using concentrations of 1.5–2.3 g/100 ml. Intensities of infrared absorption are indicated in parentheses after the frequencies either by the semiquantitative notation used previously (13), or as molecular extinction coefficients. Chromatography was on Woelm alumina; Brockmann activity grades shown.

4-Oxa-5α-cholestan-3α-ol (Va)

Lithium aluminum hydride (215 mg, 5.41 mmoles) in anhydrous, peroxide-free tetrahydrofuran (70 ml) was added under nitrogen to a stirred solution of 4-oxa-5α-cholestan-3-one (7.50 g, 19.33 mmoles) in the same solvent (75 ml), over a period of 10 minutes. During the addition, the reaction flask was cooled in an ice–salt bath; it was then allowed to warm up to room temperature while the stirring was continued for 1 hour. The reaction mixture was poured into cold 1 *N* sulphuric acid (150 ml). The white solid which precipitated was removed by filtration, washed with water, and dried. It was

taken up in anhydrous ethyl acetate and filtered through infusorial earth. Addition of petroleum ether to the filtrate resulted in the separation of colorless crystals (6.32 g, 84% yield), m.p. 184°–186° C. The melting point was raised, by recrystallization, to 188°–190° C (bath preheated to 180°, temperature rise 2° C per minute), but was dependent on the rate of heating. $[\alpha]_D^{25}$ +64° (in chloroform); $[\alpha]_D^{26}$ +88° (in tetrahydrofuran); $\nu_{max}^{CCl_4}$ in cm^{-1}: 3620 (55), 3385 (60), 2928 (495), 2853 (290), 1469 (152), 1449 (135), 1385 (115), 1368 (110), 1083 (285), 1045 (220), 947 (118). Calc. for $C_{26}H_{46}O_2$: C, 79.94; H, 11.87%. Found: C, 79.95; H, 12.02%.

3ξ-2′,4′-Dinitrophenylhydrazo-4-oxa-5α-cholestane (VIII)

A solution of 4-oxa-5α-cholestan-3α-ol (78 mg) in 96% ethanol (6 ml) was added to a solution of 2,4-dinitrophenylhydrazine (39.6 mg) in 96% ethanol (2 ml) containing concentrated hydrochloric acid (0.8 ml). The mixture was boiled for 10 minutes. When the solution was cooled, yellow crystals (71 mg) separated, which after two recrystallizations from ethanol–benzene (9:1, v/v) melted at 154.5°–155.5° C. $[\alpha]_D^{25}$ +3.2° (*c*, 2.36 in chloroform); $[\alpha]_D^{23}$ +7.6° (*c*, 1.58 in pyridine–methanol (1:1, v/v); $\lambda\lambda_{max}$ 262, 347 mμ, $\epsilon\epsilon_{max}$ 11,900, 22,800 (in chloroform); $\lambda\lambda_{max}$ 248–255 (flat peak), 328 mμ (shoulder at 367 mμ), $\epsilon\epsilon_{max}$ 10,900, 18,500 (in cyclohexane); $\nu_{max}^{CCl_4}$ in cm^{-1}: 3350 (95), 3092 (39), 2930 (370), 2850 (200), 1620 (510), 1470 (140), 1430 (195), 1338 (610), 1313 (340), 1280 (160), 1137 (180), 1072 (235), 1062 (220), 1050 (187), 926 (130). Calc. for $C_{32}H_{50}O_5N_4$: C, 67.34; H, 8.83; N, 9.82%. Found: C, 67.45; H, 8.75; N, 10.04%.

3,5-Seco-A-norcholestan-3,5β-diol (IX)

A solution of 4-oxa-5α-cholestan-3α-ol (200 mg) in tetrahydrofuran (5 ml) was added dropwise, with stirring, under nitrogen, to a solution of lithium aluminum hydride (13 mg) in tetrahydrofuran (3 ml). The mixture was stirred a further 1.5 hours, and then the excess hydride decomposed by slowly adding aqueous tetrahydrofuran. The reaction mixture was added to ice-cold 1 *N* sulphuric acid solution. An oil separated which soon solidified (176 mg, 86%), and was identified as 3,5-seco-A-norcholestan-3,5β-diol (IX) (13) by melting point, mixed melting point, and infrared spectrum.

Reduction Product A

4-Oxa-5α-cholestan-3-one (4.33 g) in diethyl ether (75 ml) was reduced with a slurry of lithium aluminum hydride (175 mg) in diethyl ether (30 ml), following the procedure described above for 4-oxa-5α-cholestan-3α-ol. Instead of a white solid, a syrup was obtained, which was chromatographed on alumina (Merck). Elution with the solvents indicated gave the following fractions: (1) a syrup (2.22 g), shown by infrared analysis to contain no hydroxyl group (ligroin); (2) 4-oxa-5α-cholest-2-ene (23 mg), identified by infrared spectrum (ligroin); (3) 4-oxa-5α-cholestan-3α-ol, isolated after several recrystallizations from ethyl acetate as needles (280 mg), m.p. 184–186°, and identified by mixed melting point and infrared spectrum (benzene–ether (1:1, v/v)); (4) product A (ether); (5) 3,5-seco-A-norcholestan-3,5β-diol, isolated after several recrystallizations from ethyl acetate as needles (65 mg), m.p. 133–134°, and identified by mixed melting point and infrared spectrum (ether–ethanol (1:1, v/v)).

Product A crystallized from anhydrous ethyl acetate as colorless needles (309 mg), m.p. 139.5°–140.5° C. $[\alpha]_D^{26}$ +56.5 (*c*, 2.66 in chloroform); $[\alpha]_D^{27}$ +54.9 (*c*, 1.98 in tetrahydrofuran); $\nu_{max}^{CCl_4}$ in cm^{-1}: 3580 (m), 3470 (m), 2960 (s), 2885 (s), 1473 (s), 1452 (s), 1387 (s), 1371 (m), 1347 (w), 1127 (m), 1036 (s), 933 (w). Calc. for $C_{78}H_{140}O_5$: C, 80.90; H, 12.18%; molecular weight, 1158. Found: C, 80.75; H, 12.17%; molecular weight (Rast, camphor), 590.

Reaction of Product A with Methanolic Hydrogen Chloride

Acetyl chloride (0.6 ml) was added to absolute methanol (15 ml) to give an approximately 3% solution of hydrogen chloride. Product A (150 mg) was dissolved in this solution, and the mixture refluxed for 1 hour and then concentrated by distillation to 7 ml. The solution, on cooling, deposited plates (44 mg), m.p. 86°–88° C, raised to 98°–99.5° by recrystallization from methanol, which were identified as 3α-methoxy-4-oxa-5α-cholestane (see below) by mixed melting point, specific rotation, and infrared spectrum.

The mother liquors from the first crystallization were taken to dryness *in vacuo*, and the solid thus obtained was washed with water and dried. The crude solid (97 mg, m.p. 115°–121° C), after recrystallization from ligroin, melted at 134°–134.5° C, and was identified as 3,5-seco-A-norcholestan-3,5β-diol (IX) (13) by mixed melting point and infrared spectrum.

4-Oxa-5α-cholest-2-ene (X)

(*a*) A solution of 4-oxa-5α-cholestan-3α-ol (900 mg) and phosphorus oxychloride (2.3 ml) in pyridine (7 ml) was refluxed for 1 hour. The cooled solution was diluted with water and extracted with ether. The ether solution was washed with aqueous bicarbonate, dried (sodium sulphate), concentrated, and diluted with methanol (10 ml). On standing, the solution deposited colorless prisms of 4-oxa-5α-cholest-2-ene (686 mg, 80% yield), m.p. 68–70° C, raised by recrystallization from ethanol to 70°–72° C. $[\alpha]_D^{25}$ +88.8° (chloroform); ϵ_{210} 5500 (in cyclohexane, end absorption only); $\nu_{max}^{CCl_4}$ in cm^{-1}: 3050 (40), 2930 (510), 1647 (133), 1470 (150), 1448 (120), 1386 (110), 1370 (79), 1243 (95), 1204 (82), 1080 (420), 945 (82), 914 (40). Calc. for $C_{26}H_{44}O$: C, 83.82; H, 11.91%. Found: C, 83.83; H, 12.00%.

(*b*) A solution of 4-oxa-5α-cholestan-3α-ol (300 mg) and fused sodium acetate (150 mg) in acetic anhydride (2.0 ml) was refluxed for 5 hours, and then poured into ice water. The brown oil separating slowly solidified, and was purified by several crystallizations from methanol, giving white plates, m.p. 67–68°, and shown by mixed melting point and infrared spectrum to be identical with 4-oxa-5α-cholest-2-ene obtained above.

4-Oxa-5α-cholestane (XIII)

4-Oxa-5α-cholest-2-ene (510 mg) in glacial acetic acid (20 ml) was hydrogenated over platinum oxide (140 mg). After 3 hours, 95% of the theoretical volume of hydrogen had been absorbed. The catalyst was removed by filtration, the solution diluted with ether and washed with water, aqueous bicarbonate, and water, and dried (sodium sulphate). Evaporation of the ether left a white solid (0.48 g), which after one crystallization from methanol had m.p. 89°–90° C, undepressed by admixture with 4-oxa-5α-cholestane prepared from 3,5-seco-A-norcholestan-3,5β-diol (13), and having the same infrared spectrum. Fieser *et al.* (18) have reported a melting point of 93°–94° C for this compound prepared by a different route.

3-Acetoxy-4-oxa-5α-cholestane (XI, R = MeCO)

4-Oxa-5α-cholestan-3α-ol (204 mg) was dissolved in pyridine (3 ml) with gentle warming, and acetic anhydride (0.3 ml) was added to the cooled solution. After 2 days, the solution was poured into ice water to give a solid which was washed free from acid with water and dried. The crude product (203 mg), m.p. 90°–101° C, was probably a mixture of the isomeric 3-acetoxy compounds (XI*a* and XI*b*, R = MeCO); $\nu_{max}^{CCl_4}$ in cm^{-1}: 2940 (s), 2860 (s), 1755 (s), 1473 (m), 1463 (sh), 1450 (m), 1385 (m), 1374 (m), 1222 (s), 1158 (m),

1142 (m), 1046 (s), 939 (m), 891 (m). Recrystallization from methanol afforded a mixture (164 mg), m.p. 65–80° C, as shown by chromatography on neutral alumina (5 g, grade III). The following fractions were eluted: (*a*) with hexane (30 ml), 3α-methoxy-4-oxa-5α-cholestane (72 mg), m.p. and mixed m.p. 93°–94° and 94°–95°; (*b*) with hexane–benzene, 1:1 (30 ml), a fraction (62 mg), m.p. 74°–85° C, having the same infrared spectrum as the crude acetate described above; (*c*) with ether (50 ml), 4-oxa-5α-cholestan-3α-ol (20 mg) identified by infrared spectrum. Attempts to recrystallize the acetate from anhydrous ether, tetrahydrofuran, benzene, or petroleum ether failed.

3α-Chloro-4-oxa-5α-cholestane (XIV)

A solution of 4-oxa-5α-cholest-2-ene (140 mg) in anhydrous ether (10 ml) saturated with hydrogen chloride was kept under nitrogen for 2 hours. Evaporation of the ether by a jet of dry nitrogen left crystals which sintered at 95° C, m.p. 104°–107° C. $[\alpha]_D^{20}$ +156° (*c*, 1.56 in absolute chloroform); $\nu_{max}^{CCl_4}$ in cm^{-1}: 2925 (585), 2855 (330), 1469 (150), 1448 (135), 1384 (110), 1367 (92), 1330 (47), 1155 (272), 1130 (292), 1119 (sh, 197), 1033 (258), 928 (82), 908 (56). Calc. for $C_{26}H_{45}OCl$: C, 76.34; H, 11.09%. Found: C, 76.16; H, 10.89%. Quantitative infrared measurements showed that this material, in different preparations, contained 3–6% of starting material; however, all attempts at recrystallization led to a lowering of the melting point.

3α-Methoxy-4-oxa-5α-cholestane (XI, R = Me)

(a) From 4-Oxa-5α-cholestan-3α-ol

Acetyl chloride (1.2 ml) was added to absolute methanol (30 ml) to give an approximately 3% solution of hydrogen chloride. A solution of 4-oxa-5α-cholestan-3α-ol (333 mg) in this solvent was refluxed for 30 minutes. When concentrated to 10 ml, the solution deposited glistening white plates (302 mg), m.p. 91–93° C. These were washed with water to remove traces of acid, dried, and recrystallized from methanol, giving crystals melting at 98°–99.5° C. $[\alpha]_D^{26}$ +109° (*c*, 1.43 in chloroform); $[\alpha]_D^{22}$ +113.5° (*c*, 1.49 in methanol–tetrahydrofuran (1:1, v/v)); $\nu_{max}^{CCl_4}$ in cm^{-1}: 2930 (580), 2860 (340), 1470 (170), 1457 (138), 1448 (148), 1382 (124), 1368 (134), 1132 (360), 1058 (360), 1039 (412), 934 (105), 893 (178). Calc. for $C_{27}H_{48}O_2$: C, 80.20; H, 11.95; OMe, 7.68%. Found: C, 80.25; H, 12.00; OMe, 7.92%.

Fieser *et al.* (18) have recently reported preparing, by a different route, a compound, m.p. 89°–92° C, having the structure of our compound but undefined stereochemistry; it is probably identical with our compound.

(b) From 3α-Chloro-4-oxa-5α-cholestane

Sodium (60 mg) was dissolved in absolute methanol (12 ml), followed by 3α-chloro-4-oxa-5α-cholestane (153 mg), dissolution of the latter requiring that the solution be warmed. The solution was refluxed for 40 minutes, and the solvent then removed under reduced pressure. The residue was treated with water (12 ml) and ether (12 ml), and the ether layer was washed, dried, and diluted with methanol (8 ml). On standing for 2 days, with partial evaporation of the ether, the solution deposited long plates of 3α-methoxy-4-oxa-5α-cholestane (110 mg), m.p. 98.5–99.5, undepressed by admixture with authentic material. Further concentration of the solution gave a brownish semisolid mass (48 mg), which in carbon tetrachloride solution had an infrared spectrum similar to that of pure 3α-methoxy-4-oxa-5α-cholestane, with the addition of a peak at 1068 cm^{-1} and a shoulder at 1126 cm^{-1}. This material resisted all attempts at purification.

(c) From 3α-Chloro-4-oxa-5α-cholestane in the Presence of Silver Carbonate

To a suspension of powdered calcium chloride (500 mg) and freshly prepared silver carbonate (53) (230 mg) in absolute chloroform (15 ml) was added a solution of 3α-chloro-4-oxa-5α-cholestane (287 mg) in absolute chloroform (15 ml), followed by the slow addition, with vigorous stirring, of methanol (0.12 ml) in absolute chloroform (3 ml). The mixture was stirred for 30 minutes at room temperature and 30 minutes at 50° C. Then water was added, the mixture was filtered through infusorial earth, and the organic layer was separated, washed with water, and dried. Concentration of this solution afforded a syrup which in contact with a little methanol solidified. The solid, crystallized from ether–methanol, gave a first crop (226 mg), m.p. 86°–94° C, $[\alpha]_D^{20}$ +110° (*c*, 1.15 in chloroform); second crop (15 mg), m.p. 79°–85° C, $[\alpha]_D^{20}$ +98.5° (*c*, 1.16 in chloroform); third crop (7 mg), m.p. 62°–68° C; and a brownish semisolid residue (28 mg). Infrared examination showed the first crop to be 3α-methoxy-4-oxa-5α-cholestane contaminated with a small amount of 4-oxa-5α-cholest-2-ene, and the third crop to have the spectrum of 3α-methoxy-4-oxa-5α-cholestane, with the addition of a weak shoulder at 1068 cm^{-1}.

Hydrolysis of 3α-Methoxy-4-oxa-5α-cholestane

A solution of 3α-methoxy-4-oxa-5α-cholestane (150 mg) in tetrahydrofuran (10 ml) – 15% aqueous hydrochloric acid (3.5 ml) was refluxed for 10 minutes, then poured into ice water. A solid was removed by filtration, washed with water, and dried; this crude product (125 mg), m.p. 154°–158° C, was recrystallized from methanol and identified as 4-oxa-5α-cholestan-3α-ol by melting point, mixed melting point, and infrared spectrum.

3α- and 3β-Benzyloxy-4-oxa-5α-cholestane (XIa and XIb, R = CH_2Ph)

(a) From 4-Oxa-5α-cholestan-3α-ol

Acetyl chloride (0.4 ml) was added to benzyl alcohol (10 ml), then 4-oxa-5α-cholestan-3α-ol (100 mg) was dissolved in this solution. The solution, after 24 hours at room temperature, was concentrated at 0.5 mm; removal of the dibenzyl ether formed in this reaction required that the flask be heated to 100°. The residual syrup, which partially crystallized, was chromatographed on basic alumina (25 g, grade III), and the fractions eluted from the column were examined by infrared spectroscopy. The fractions fell into the following groups: (*a*) with ligroin (b.p. 65–75°; 60 ml), 4-oxa-5α-cholest-2-ene, obtained as crystals (10 mg), m.p. 63–66°, having the characteristic infrared spectrum; (*b*) with more ligroin (110 ml), a syrup (65 mg) identified as 3α-benzyloxy-4-oxa-5α-cholestane (see below); (*c*) with ligroin–benzene (4:1, v/v) (30 ml), a syrup (25 mg) shown by infrared spectroscopy to be a slightly impure mixture of 3α- and 3β-benzyloxy-4-oxa-5α-cholestane; (*d*) with more ligroin–benzene (4:1, v/v) (50 ml), impure crystals of 3β-benzyloxy-4-oxa-5α-cholestane.

Fractions (*b*) were dissolved in hot methanol and filtered through charcoal–celite. The syrup (62 mg) which separated on being cooled was dried at 100° C at 0.5 mm. The syrup from some runs crystallized to a solid, m.p. 42–51° C, when cooled to 15° and rubbed; the syrup from other runs could not be crystallized, but was indistinguishable with respect to specific rotation and infrared spectrum. Attempts to recrystallize the solid at low temperatures with seeding afforded always a syrup without change in specific rotation or infrared spectrum: $[\alpha]_D^{26}$ +99° (*c*, 2.23 in chloroform); $[\alpha]_D^{22}$ +91° (*c*, 1.46 in 3:2 benzyl alcohol – tetrahydrofuran, v/v); $\lambda\lambda_{max}^{EtOH}$ 248, 252.5, 258, 264, 268 mμ; $\epsilon\epsilon_{max}$ 165, 234, 272, 217, 148; $\nu_{max}^{CCl_4}$ in cm^{-1}: 3060 (40), 3020 (55), 2920 (540), 2855 (355),

1470 (175), 1457 (175), 1382 (128), 1342 (88), 1164 (100), 1130 (250), 1052 (215), 1037 (380), 1027 (360), 945 (110), 935 (125), 889 (90), 696 (81). Calc. for $C_{33}H_{52}O_2$: C, 82.45; H, 10.91%. Found: C, 82.37; H, 10.84%.

Chromatographic fractions (*d*) were dissolved in ligroin and treated with charcoal. Evaporation of the solvent afforded well-formed prisms, which were washed on the filter with ligroin (cooled to −10° C) and then recrystallized from ligroin or methanol to give pure 3β-benzyloxy-4-oxa-5α-cholestane (12 mg), melting at 131°–132° C. $[\alpha]_D^{22}$ −22° (*c*, 2.02 in chloroform; $[\alpha]_D^{22}$ −3.8 (*c*, 1.46 in tetrahydrofuran – benzyl alcohol (2:3, v/v)); $\lambda\lambda_{max}$ 248, 252.5, 258.5, 264.5, 268 mμ; $\epsilon\epsilon_{max}$ 171, 218, 252, 210, 151 (in cyclohexane); $\nu_{max}^{CCl_4}$ in cm^{-1}: 3060 (40), 3028 (57), 2930 (575), 2850 (340), 1497 (40), 1469 (174), 1457 (180), 1450 (155), 1387 (134), 1370 (126), 1344 (102), 1166 (224), 1141 (210), 1120 (215), 1094 (190), 1070 (510), 1047 (335), 935 (122), 902 (66), 695 (88). Calc. for $C_{33}H_{52}O_2$: C, 82.45; H, 10.91%. Found: C, 82.38, H, 10.98%.

(b) From 3α-Chloro-4-oxa-5α-cholestane

A suspension of 3α-chloro-4-oxa-5α-cholestane (265 mg) and finely powdered anhydrous sodium acetate (150 mg) in anhydrous benzyl alcohol (5 ml) was heated on a steam bath for 1 hour. The solvent was then removed at reduced pressure, the residue dissolved in ether and water, and the ether layer dried and then taken to dryness. The residue was dissolved in hexane and chromatographed on basic alumina (grade III), as described above, to give slightly impure 3α-benzyloxy-4-oxa-5α-cholestane (239 mg, 76%) and 3β-benzyloxy-4-oxa-5α-cholestane (70 mg, 22%), identified by melting point and infrared spectra.

Isomerization of 3α-Benzyloxy-4-oxa-5α-cholestane (XIa, R = CH_2Ph)

Acetyl chloride (4 drops) was added to benzyl alcohol (0.4 ml) to generate hydrogen chloride, and the solution was diluted with anhydrous ether (3 ml). 3α-Benzyloxy-4-oxa-5α-cholestane (110 mg) was dissolved in this solution. After 4 hours at room temperature, the solvents were removed at reduced pressure (0.5 mm at 80° C) and the residual syrup was chromatographed on basic alumina (grade III) as described above to give starting material (71 mg) and 3β-benzyloxy-4-oxa-5α-cholestane (13 mg), identified by melting point, mixed melting point, and infrared spectrum.

For preparative purposes, the yield of β isomer was increased by allowing a syrupy mixture of isomers containing a trace of acid to stand for several weeks, after which time a certain amount of the β isomer had crystallized out. The whole mixture was then neutralized and chromatographed to give a 25% yield of β isomer.

Action of Titanium Tetrachloride on 3α-Benzyloxy-4-oxa-5α-cholestane

Titanium tetrachloride was purified by standard vacuum-line techniques, and an estimated 100–150 mg was distilled into a frozen solution of 3α-benzyloxy-4-oxa-5α-cholestane (120 mg) in carbon tetrachloride (5 ml). The mixture was allowed to melt under vacuum, refluxed for 5 hours with rigid exclusion of moisture, and then poured into ice water. More carbon tetrachloride was added and the organic layer was separated, washed with bicarbonate and water, and dried over magnesium sulphate. Removal of the solvent at reduced pressure left a yellowish solid (125 mg) which was treated with cold ligroin. The undissolved fraction (35 mg) proved by melting point and mixed melting point to be slightly impure 4-oxa-5α-cholestan-3α-ol. The ligroin solution, on chromatography on basic alumina (grade III), gave only glassy, unidentifiable materials, followed by a small quantity of impure 4-oxa-5α-cholestan-3α-ol (18 mg).

17α-Oxa-D-homo-5α-androstan-3β-ol (XXIV) (40)

13,17-Seco-5α-androstan-3β,13α-17-triol (54) (510 mg) was treated with benzenesulphonyl chloride in lutidine under conditions already described (13). The product, isolated in the usual way, was an oil (427 mg) which was chromatographed on alumina (activity II–III). Elution with ether–acetone, 9:1, gave 17α-oxa-D-homo-5α-androstan-3β-ol (114 mg), m.p. 171°–174° C, raised by one crystallization from methanol to 174°–175° C. $\nu_{max}^{CCl_4}$ in cm^{-1}: 3635 (m), 2940 (s), 2860 (sh), 2708 (w), 1462 (sh), 1451 (s), 1440 (sh), 1375 (s), 1282 (m), 1212 (w), 1155 (m), 1132 (sh), 1120 (s), 1080 (s), 1075 (sh), 1064 (sh), 1044 (s), 1032 (s), 973 (w), 955 (w), 945 (w), 935 (m), 923 (w), 895 (w), 877 (w), 848 (w), 840 (w). Calc. for $C_{19}H_{32}O_2$: C, 78.02; H, 11.03%. Found: C, 77.84; H, 10.79%.

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LYCOPODIUM ALKALOIDS

X. THE STRUCTURE OF LYCOPODINE[1]

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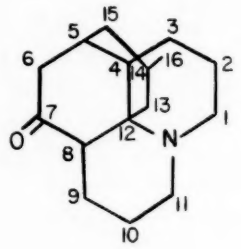
ABSTRACT

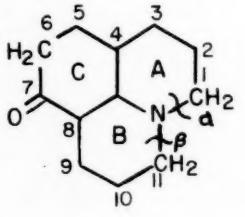
The reactions leading to the establishment of structure I for lycopodine are discussed. Structures are proposed for three cyclization products derived from lycopodine.

In a short communication (1) we proposed structure I for lycopodine. This structure has been confirmed by the work of Anet (2), who succeeded in interconverting several *Lycopodium* alkaloids, among which was lycopodine. In this paper we wish to give a full account of our work on the elucidation of the structure of the alkaloid and to discuss the structures of the tetracylic compounds obtained on elimination of hydrogen halide from lycopodine methiodide and α- and β-cyanobromolycopodine.



I

Partial structure A proposed in 1959 (3) is a convenient starting point for the ensuing discussion.



A

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[2]*Holder of a National Research Council Studentship, 1956–59.*
[3]*Holder of a National Research Council Bursary, 1958–59, and a National Research Council Studentship, 1959–60.*

In structure A the carbon atoms known with certainty at that time are designated, as are the bonds cleaved in the formation of α- and β-cyanobromolycopodine, II and III respectively (4, 5). The reasons for proposing structure A are reviewed briefly below. Entry had been gained to rings A and B by reaction with cyanogen bromide. The α-bromide II had been converted to the corresponding acid and thence to the ring-A lactam IV. The infrared spectrum of the latter indicated that ring A was six-membered or larger (3). The β-bromide III had been converted to the corresponding acid which, on reduction with sodium borohydride, readily formed a lactone (V). The infrared spectrum of the lactone indicated that it was either five- or six-membered (3). The carbonyl ring, C, was considered to be six-membered or larger because of the infrared absorption of the carbonyl group near 1700 cm^{-1} (4). The presence of the methylene group adjacent to the carbonyl had been established by conversion of α-cyanolycopodine (VI) to a benzylidene derivative (VII) and to an enolic α-diketone (VIII) (5). Finally, the hexahydrojulolidine system had been found in the alkaloid annotinine, then the only alkaloid of the *Lycopodium* group of known structure (6). No attempt was made at that time to locate the fourth ring of lycopodine.

Support for the proposal that ring B was six-membered came from the following sequence. The methyl ester, IX, of β-cyanolycopodine carboxylic acid (3) was converted by hydrolysis and subsequent treatment with diazomethane to the methyl ester of the corresponding amino acid. When the ester was heated under reflux in xylene it yielded the lactam X, $C_{16}H_{23}O_2N$, which showed absorption in the infrared at 1626 cm^{-1} indicative of a six-membered or larger lactam. Reduction of X with lithium aluminum hydride gave dihydrolycopodine XI (4). Thus no skeletal rearrangement had occurred in the reactions outlined.

The nature of four more carbon atoms in rings A and B was established by modified Kuhn–Roth oxidation of α- and β-cyanodihydrolycopodine, XII and XIII respectively (5, 7). The oxidations were performed according to the procedure of Lemieux and Purves (8). The volatile acids were recovered from the aqueous distillate as their sodium salts. The free acids were liberated with dry hydrogen chloride, distilled, and converted to their methyl esters with diazomethane. The esters were separated by liquid–vapor partition chromatography and identified by comparison of their retention times with values determined by running known mixtures under the same conditions.

Both XII and XIII yielded mixtures of acetic, propionic, and *n*-butyric acids, and it can be concluded that both compounds contain *n*-propyl groups attached to carbon. Oxidation of lycopodine under the same conditions yielded only acetic acid. Therefore, methylene groups can be written at C_2, C_3, C_9, and C_{10} in structure A. The relatively high proportion (24%) of butyric acid in the acid mixture derived from the oxidation of XII suggests that C_4 is not quaternary which implies that the carbon skeleton of lycopodine must differ from that of annotinine. These results establish the sequence $-(CH_2)_3-N-(CH_2)_3-$ in the lycopodine molecule and are consistent with structure A, but do not verify the proposed relationship between the carbonyl function and the nitrogen atom. However, the reactions of the carbonyl ring reported below have established that the proposed relationship is the correct one.

Partial structures for some of the above compounds are given in Chart I.

The benzylidene derivative, VII, of α-cyanolycopodine was the key compound in the investigation of ring C. Upon ozonolysis it yielded the enolic diketone VIII, which had been prepared previously by Barclay and MacLean (5) by bromination and hydrolysis

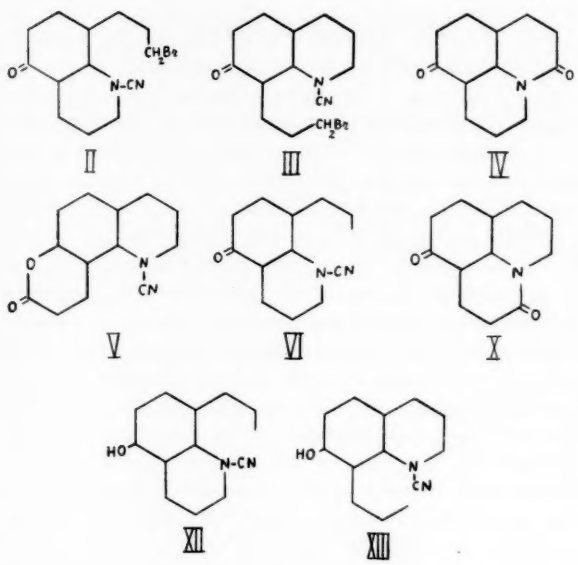


CHART I

of α-cyanolycopodine (VI). The enolic character of this α-diketone established that at least one enolizable hydrogen must be present on a carbon adjacent to one of its two carbonyl groups. The alternatives are represented in partial formulas VIII(a) and VIII(b).

Oxidation of VII with selenium dioxide yielded two products. One of these products was a compound (XIV), $C_{24}H_{30}O_2N_2$, which had hydroxyl absorption in its infrared spectrum at 3560 cm^{-1} and carbonyl absorption at 1692 cm^{-1} in chloroform solution. The carbonyl absorption of the starting material VII appeared at 1685 cm^{-1} in the same solvent. This shift in the carbonyl frequency implied that the hydroxyl group had entered alpha and axial to the carbonyl function. The second oxidation product (XV), $C_{24}H_{28}ON_2$, was a yellow compound formed from the starting material by loss of two hydrogens. The ultraviolet spectrum of XV differed markedly from that of both VII and XIV, in each of which a maximum occurred near 280 mμ. In XV the 280-mμ band of the starting material had shifted to 310 mμ. The infrared spectrum of XV was also very different from that of VII. There were three very sharp bands at 1677 cm^{-1}, 1626 cm^{-1}, and 1590 cm^{-1} when the spectrum was run in chloroform. The shift in the frequency of the carbonyl band from 1685 in VII to 1677 cm^{-1} in XV indicated that reaction had occurred adjacent to the carbonyl group. The band at 1626 cm^{-1} is assigned to olefin absorption and that at 1590 cm^{-1} to the conjugated phenyl group. The analysis and the spectral data are compatible with the presence, in the molecule, of the chromophoric system

$$C_6H_5CH{=}\overset{\overset{O}{\|}}{\underset{|}{C}}{-}\overset{|}{C}{-}\overset{|}{C}{=}C{-}.$$

The spectrum of an analogous system has been studied in

the infrared (9) and in the ultraviolet region (10) and the results are similar to, although not identical with, the above. The formation of XV from VII occurred without alteration of the carbon skeleton since both VII and XV yielded the same product (XVI), $C_{24}H_{32}ON_2$, on treatment with hydrogen over a platinum catalyst.

Ozonolysis of XIV gave two products, a diketo alcohol (XVII), $C_{17}H_{24}O_3N_2$, and a lactone carboxylic acid (XVIII), $C_{17}H_{24}O_4N_2$. The former was a yellow compound with weak absorption in the ultraviolet at 420 mμ characteristic of an α-diketone. Treatment of XVII with H_2 and platinum yielded the enol VIII. However, unlike VIII, compound XVII had no enolic properties. One must therefore conclude that the hydroxyl group in XVII has replaced the only enolizable hydrogen in VIII and that the carbon adjacent to one of the two carbonyl groups is quaternary or at a bridgehead where enolization would be in violation of Bredt's rule.

Deuterium exchange of lycopodine (I) and α-cyanolycopodine (VI) established the presence of three enolizable hydrogens in each. Therefore, partial structure VIII(a) must represent enol VIII and partial structures XIV, XV, XVII, and XVIII, the hydroxybenzal compound, the olefinic benzal compound, the diketo alcohol, and the lactone carboxylic acid, respectively. Therefore, position 5 in partial structure A, must be quaternary or at a bridgehead. This represents again a departure from the annotinine skeleton. Partial structures for the compounds above are given in Chart II.

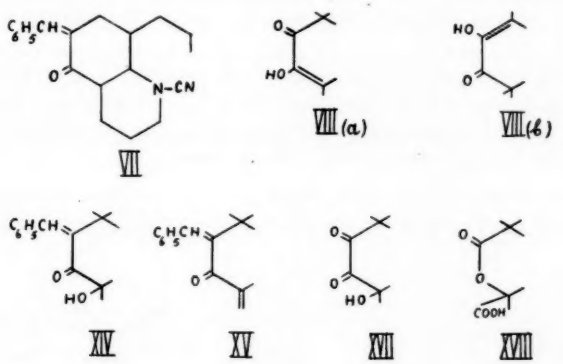
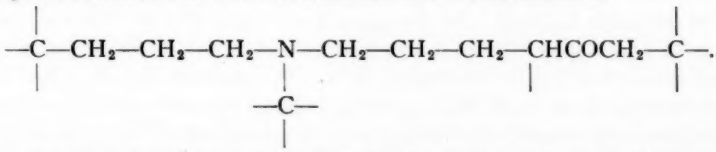


CHART II

The peripheral structure of the molecule can now be extended to



This is the only structure compatible with the formation of lactone V on borohydride reduction of the acid derived from ester IX, and with the above results.

This structure is in complete accord with partial structure A but, although the evidence

for structure A is substantial, it is not unequivocal. Rings A and B must make up a reduced quinolizine system if these rings are six-membered, as lactams IV and X suggest. There is evidence that rings B and C make up a reduced quinoline system, because dehydrogenation of XIX (a product formed by dehydration and hydride reduction of XII) yielded a base (XX), in small yield, with the properties of a quinoline. Although the structure of this base has not been proved, it may be 8-*n*-propyl-7-isobutylquinoline.

In partial structure A there are only three positions, C_4, C_5, and C_{12}, to which the fourth ring of the alkaloid, ring D, can be attached. Since it has been shown that C_5 is either quaternary or at a bridgehead and since there is evidence from chromic acid oxidation that C_4 is not quaternary, it appears that ring D is closed by a bridge between C_5 and C_{12}.

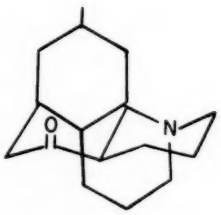
The structure of this bridge may be deduced from the dehydrogenation experiments of Marion and Manske (11). They found that lycopodine yielded five basic products on dehydrogenation, of which they were able to identify two: 7-methylquinoline and 5,7-dimethylquinoline. The tendency to form 7-methylquinoline was particularly strong. By

$$\begin{array}{c} \\ \\ \mathrm{CH_3} \\ | \\ \mathrm{CH} \end{array}$$

bridging C_5 and C_{12} in structure A with the unit $—CH_2—CH—CH_2—$, there is obtained structure I. This structure is completely consistent with the dehydrogenation results and is compatible with the rest of the chemical evidence. It is also in accord with the N.M.R. spectrum of lycopodine, which shows a single C-methyl group attached to a tertiary carbon.

The relative configuration of the asymmetric centers in lycopodine has been discussed by Anet (2). Except for the configuration at C_{14}, for which we had no experimental evidence, we had arrived at the same conclusion (12). The perspective formulas B and C give an idea of the spatial relationships which exist.

B

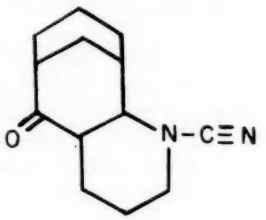


C

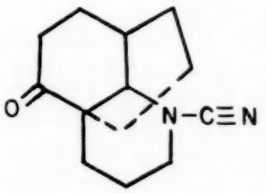
The elucidation of the structure of lycopodine has at the same time provided the structures of alkaloids L 2 and L 14. Douglas, Lewis, and Marion (13) have shown that L 2 is the acetyl derivative of dihydrolycopodine and that L 14 is anhydrodihydrolycopodine. The double bond in L 14 occupies the C_7—C_8 position, for Anet has shown, and we have confirmed, that its N.M.R. spectrum shows the presence of a single olefinic proton. We have also shown that alkaloid L 1, complanatine (14), is identical with dihydrolycopodine; its formula should be revised to $C_{16}H_{27}ON$. The formation of dihydrolycopodine from lycopodine by hydride reduction implies that the OH group

is axial in the chair conformation and cis to the C_5—C_{12} bridge since hydride reduction will occur from the less-hindered side of the system.

The structure of the cyclized product XXI, $C_{17}H_{24}ON_2$, formed from α-cyanobromolycopodine by treatment with alkali (4), has now been clarified. Recently (7) we proposed the alternative partial structures D and E for this compound. Deuterium exchange



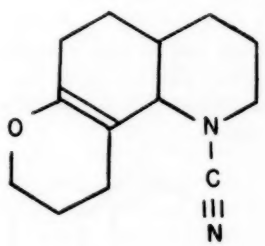
D



E

studies and infrared spectroscopy have allowed us to differentiate between the alternatives. It was found that XXI exchanged two hydrogens for deuterium and this is consistent with partial structure E. Such a result is possible but unlikely for the bicyclo-(3.3.1)nonane system present in D. In the infrared spectrum of XXI there is a band at 1413 cm^{-1} which is absent in deuterated XXI. This behavior is in keeping with ketones containing an α-methylene group (15, 16). Therefore partial structure E must pertain. It is the study of this cyclization reaction which has allowed an assignment of configuration to C_4 of the lycopodine molecule and the other alkaloids which Anet (2) has related to lycopodine.

An isomeric cyclized compound (XXII), $C_{17}H_{24}ON_2$, is obtained from the β-bromide III by treatment with base (4), and for this compound we proposed partial structure F (7).



F

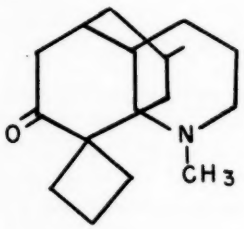
This compound does not exchange hydrogen for deuterium, in keeping with the proposed structure. Its N.M.R. spectrum showed two groups of peaks which were distinctly separate from the bulk of the absorption. The areas under the curves corresponded in both cases to two protons. One group of peaks ($\delta = 2.36$) was in the region characteristic of

protons in a $—CH_2—O—$ group while the other (δ = 4.14) was probably associated with the hydrogens of C_1, which is adjacent to the cyanamide function. No band was observed which could be assigned to a $\diagdown C{=}C—H \diagup$ group.* The N.M.R. spectrum of XXI was run for comparison, and it showed one unresolved band (δ = 4.09; area = two protons) slightly displaced from the rest of the spectrum. This band was attributed to the protons of C_{11}.

The reasons for the unusual stability of the enol ether function of XXII become clear when an examination is made of a molecular model. As can be seen from the perspective formulas of lycopodine, B and C, C_8 is particularly subject to steric hindrance. The approach of bulky reagents to the vicinity of C_8 will, therefore, be opposed. This will be generally true, not only of the alkaloid, but also of all the known derivatives. In addition, the nature of the structure is such that the $C_7—C_8$ position is a particularly favorable one for a double bond. The introduction of unsaturation at $C_7—C_8$ (or $C_8—C_9$) will relieve steric hindrance by eliminating the interaction of the C_8 hydrogen with the axial hydrogens of C_1 and C_3 as well as the interaction between the hydrogens of C_9 and C_{13}. It is to be expected that any attempt to destroy the planarity of C_8 by attack on the double bond will be resisted. Cyclized compound XXII is not the only known example of a lycopodine derivative with a very stable $C_7—C_8$ double bond. We have found that anhydrodihydrolycopodine is resistant to hydrogenation and ozonolysis.

A third cyclized compound (XXIII), $C_{17}H_{27}ON$, which has not been previously reported, has been derived from lycopodine methiodide by the action of potassium tertiary butoxide in tertiary butanol. This compound showed no double-bond absorption in its infrared and was inert to the action of ozone and hydrogen. Its N.M.R. spectrum was devoid of peaks in the region of olefinic protons. On treatment with cyanogen bromide it yielded a cyanamide (XXIV), $C_{17}H_{24}ON_2$, which was isomeric but not identical with either of the two cyclized compounds, XXI and XXII, discussed above. The oxygen function in XXIII was not affected by sodium borohydride in ethanol or by lithium aluminum hydride in ether or tetrahydrofuran. However, the infrared spectrum of XXIII showed a strong peak in the carbonyl region at 1695 cm^{-1} and there was also a peak at 1410 cm^{-1} characteristic of a $—CH_2—$ adjacent to a carbonyl function.

Deuterium exchange was carried out on this compound and it was found that two enolizable hydrogens were present. Furthermore, the absorption in the infrared at 1410 cm^{-1} was absent in the deuterated compound. The only structure consistent with these data is structure XXIII below.



XXIII

Displacements are recorded in parts per million from chloroform.

EXPERIMENTAL

Lycopodine

The alkaloid was isolated from *Lycopodium flabelliforme* by the procedure described by Barclay and MacLean (5). It melted at 116° after recrystallization from petroleum ether.

The nuclear magnetic resonance spectrum of the alkaloid showed a strong doublet, $\delta = 6.6$, whose area corresponded to three protons. This was attributed to the presence of a methyl group attached to a tertiary carbon.

Dihydrolycopodine

Dihydrolycopodine was prepared by the method of MacLean, Manske, and Marion (4). We have also isolated from *L. flabelliforme* an alkaloid identical with dihydrolycopodine. This alkaloid follows lycopodine in the chromatographic separation of the crude alkaloids (5), and appears to be the second most abundant alkaloid. We have also established (14) that complanatine, alkaloid L 1, is identical with dihydrolycopodine. Identity was established by a comparison of their infrared spectra and a mixed melting point determination. We are grateful to Dr. R. H. F. Manske for a sample of complanatine.

Reaction of Lycopodine with Cyanogen Bromide

The reaction was carried out and the bromocyanamides II and III isolated by the procedure described previously (4, 5).

Preparation of the β-Lactam

To a solution of 216 mg of ester IX* (m.p. 160–162°) (3) in *n*-propyl alcohol (2.5 ml) was added 15 ml of 2 *M* hydrochloric acid. After the mixture had been heated for 15 hours on a steam bath it was evaporated to dryness, and the residue was washed with ether to remove unhydrolyzed material. The residual crude amino acid hydrochloride was dissolved in methanol and the ice-cooled solution was treated with an excess of freshly prepared diazomethane solution. After 20 minutes the solution was evaporated and the residue worked up to give the basic product (150 mg). The infrared spectrum of the crude product showed strong ketone and ester carbonyl bands and weak absorption in the N—H region.

A solution of the crude amino ester (150 mg) in xylene (30 ml) was distilled very slowly at atmospheric pressure. After 10 hours, when the solution had been reduced to about one third of its original volume, the rest of the solvent was removed under reduced pressure. The infrared spectrum of the residual oil showed a strong band at 1625 cm^{-1} and weakened ester absorption. The oil was worked up to give basic and neutral fractions. Further purification of the neutral fraction was effected by chromatography on alumina with benzene–methanol as the eluant. A band, which passed down the column, yielded 58 mg of crystalline material. Recrystallization from ether – petroleum ether gave long, colorless needles which melted at 177–179°. Calc. for $C_{16}H_{23}O_2N$: C, 73.5; H, 8.87; N, 5.4%. Found: C, 73.5; H, 8.78; N, 5.5%.

The infrared spectrum of this compound, X, in nujol had strong maxima at 1700 cm^{-1} (ketone) and 1626 cm^{-1} (lactam carbonyl). When the spectrum was determined in chloroform solution, the bands appeared at 1709 and 1622 cm^{-1}. There was no absorption in the cyanamide and ester carbonyl regions.

A mixed melting point determination showed that lactams X and IV were not the same.

**This ester exists in two crystalline forms melting at 127–129° and 160–162°.*

Reduction of the β-Lactam with Lithium Aluminum Hydride

About 30 mg of lactam X was reduced for 20 hours with lithium aluminum hydride (0.1 g) in boiling tetrahydrofuran. The excess hydride was destroyed by the addition of wet tetrahydrofuran and the mixture evaporated. Sodium hydroxide solution was added to the residue and the organic material extracted with chloroform. Evaporation of the chloroform solution yielded an oil which partially crystallized, but attempts to obtain a sharp-melting product by recrystallization were unsuccessful. The crude product, was, therefore, dissolved in acetone and treated with methyl iodide. The crystalline methiodide which formed melted with decomposition at 287–289°. Its melting point was not depressed by the addition of dihydrolycopodine methiodide, m.p. 293–295° (decomp.). The infrared spectra of the methiodides were identical.

α-Cyanodihydrolycopodine XII

This compound was prepared by the method of Barclay and MacLean (5).

β-Cyanodihydrolycopodine XIII

This compound was prepared by the method of MacLean, Song, and Harrison (7).

Chromic Acid Oxidation of I, XII, and XIII

The oxidation procedure which was used was originally developed by Lemieux and Purves (8) for the quantitative estimation of acetyl, ethylidene, ethoxy, and α-hydroxyethyl groups. The distillate from the oxidation, which was pale yellow, was redistilled, and the colorless second distillate was titrated (pH meter) with 0.1 *N* sodium hydroxide. A small excess of alkali was added and the solution was evaporated to dryness.

Recovery of the acids was carried out by treating the residue of sodium salts with liquid hydrogen chloride. The excess hydrogen chloride was removed and the liberated acids distilled under high vacuum. The acids were recovered from their salts in about 90–95% yield by this procedure. The liberated acids contained a considerable amount of hydrogen chloride.

The recovered acids were treated with a slight excess of a freshly prepared solution of diazomethane in ether and the resulting solution of methyl esters was dried with a few crystals of anhydrous sodium sulphate. The solution was then separated into its components by gas chromatography. The methyl esters were identified by comparing the retention times of the bands with values obtained for known mixtures. The separation was carried out on a Perkin–Elmer column 'A' (didecyl phthalate on Celite) at 75° and at a flow rate of 150 ml/min. Under these conditions the following retention times (in minutes) were obtained:

Ether (used as reference)	0.00	Methyl butyrate	10.7
Methyl acetate	1.2	Methyl isovalerate	17.2
Methyl propionate	4.3	Methyl valerate	27
Methyl isobutyrate	6.8		

The actual retention time for ether was about 2 minutes. The relative yields of the acids formed in the oxidation were estimated by comparing the areas under the recorded ester peaks. The factors needed to correct the measured band areas for differences in the thermal conductivities of the esters were calculated from graphs obtained on chromatography of solutions of known composition. The over-all yield of acid was estimated from the amount of standard alkali which was required to neutralize the distillate from the oxidation. Since no titration blanks were run, however, the figures obtained for the over-all yields are not considered to be very reliable.

When oxidized by this procedure, both α- and β-cyanodihydrolycopodine, XII and XIII, gave a mixture of acetic, propionic, and butyric acid. Lycopodine gave only acetic acid. The yields of the acids were as follows:

	Relative yields (%)			Total yield of acid (%)
	Acetic	Propionic	Butyric	
I	100	—	—	(50)
XII	62	14	24	(50)
XIII	69	23	8	(70)

The yields listed in the table for I and XII were obtained by the oxidation of 100-mg samples with 10 ml of 30% aqueous chromium trioxide. Oxidation of this much material was found to be unnecessary and a sample size of 10–25 mg is now recommended.

Preparation of the Benzylidene Derivative VII

The benzylidene derivative was prepared by the procedure of Barclay and MacLean (5). The ultraviolet spectrum of this compound had two maxima, at 240 mμ, log ϵ = 4.3, and at 280 mμ, log ϵ = 4.14.

Preparation of the Enol VIII

A solution of VII (0.40 g) in 15 ml of glacial acetic acid and 15 ml of methanol was cooled to −40° in a dry ice – acetone bath. A stream of oxygen containing 5% ozone was passed through the solution for 15 minutes at a rate of 50 ml/min. The solution was then transferred to a hydrogenation bottle, platinum oxide catalyst was added, and the mixture was shaken for about 2 hours under hydrogen at a pressure of 30 p.s.i. The solution was then filtered and evaporated, dilute aqueous bicarbonate and ether were added to the residue, and the ether layer was separated. The ether solution was washed successively with dilute hydrochloric acid, sodium bicarbonate solution, and water, and then extracted with a solution of sodium hydroxide. The alkaline extract was acidified with hydrochloric acid and extracted with chloroform. Evaporation of the chloroform solution gave a crystalline product (0.25 g) which melted at 157–158° after recrystallization from ether – petroleum ether. The melting point is the same as that reported by Barclay and MacLean (4), and the melting point of a mixture of the two compounds was not depressed.

Treatment of the Benzylidene Compound VII with Selenium Dioxide

The benzylidene derivative VII (2.86 g) and selenium dioxide (1.09 g) were dissolved in 100 ml of dioxane and heated under reflux for 3 hours. The mixture was evaporated to dryness and the residue exhaustively extracted with ether. The ether extract was washed with water to remove excess selenium dioxide, dried, and concentrated. From the concentrated solution, 1.78 g of the hydroxy derivative XIV separated. This material, after recrystallization from ether, melted at 212–217° C. Calc. for $C_{24}H_{30}O_2N_2$: C, 76.1; H, 7.99; N, 7.4%. Found: C, 76.1; H, 7.90; N, 7.4%.

The infrared spectrum of this compound showed hydroxyl absorption at 3560 cm^{-1}, cyanamide absorption at 2210 cm^{-1}, and carbonyl absorption at 1692 cm^{-1}, in solution in chloroform. The ultraviolet spectrum showed a maximum at 285 mμ, log ϵ = 4.2.

The mother liquor from the separation of XIV above was treated with methanol. From the ether–methanol solution, 0.46 g of compound XV separated. After recrystallization from ether–methanol it melted at 199–202°. Calc. for $C_{24}H_{28}ON_2$: C, 79.9; H, 7.84; N, 7.8%. Found: C, 79.6; H, 7.84; N, 7.7%.

The infrared spectrum of this compound showed the usual cyanamide absorption at 2220 cm^{-1}, carbonyl absorption at 1677 cm^{-1}, a strong, sharp band at 1626 cm^{-1} attributed to double-bond absorption, and a band at 1590 cm^{-1} in the region of phenyl absorption.

The ultraviolet spectrum showed two maxima, at 310 mμ, log ϵ = 4.2, and at 228 mμ, log ϵ = 3.0.

Treatment of XIV with Ozone

Compound XIV (0.36 g) was dissolved in a mixture of 20 ml of methanol and 15 ml of glacial acetic acid. The solution was cooled to −20° C and treated with a stream of 5% ozone in oxygen for a period of ½ hour. The reaction mixture was then transferred to a flask fitted for steam distillation, and steam was passed through for 5–10 minutes. A yellow crystalline solid separated when the aqueous acetic acid mixture was cooled. The solid was collected and recrystallized from ether, and melted at 223–225° with decomposition. Calc. for $C_{17}H_{24}O_3N_2$: C, 67.1; H, 7.95; N, 9.2%. Found: C, 67.1; H, 7.99; N, 8.8%.

The infrared spectrum showed the usual cyanamide absorption at 2200 cm^{-1}, broad hydroxyl absorption and carbonyl absorption at 1724 cm^{-1}. The ultraviolet spectrum had a maximum at 420 mμ, log ϵ = 25. There was no change in the ultraviolet spectrum when the solution was made alkaline.

The mother liquor from the separation of XVII above was extracted with chloroform. The chloroform extract was extracted with aqueous bicarbonate, and the bicarbonate extract was acidified and extracted again with chloroform. The latter chloroform extract was dried over sodium sulphate and evaporated to dryness. The residue was dissolved in a small volume of acetone from which it crystallized. After recrystallization from ether–acetone, compound XVIII melted at 206–208°. Calc. for $C_{17}H_{24}O_4N_2$: C, 63.7; H, 7.55; N, 8.7%. Found: C, 63.7; H, 7.71; N, 8.4%.

The infrared spectrum of this compound showed cyanamide absorption at 2210 cm^{-1}, a peak of 1735 cm^{-1} attributed to a six-membered lactone, and a peak at 1680 cm^{-1} attributed to a carboxyl group.

Conversion of XVII to VIII

The hydroxydione XVII (0.1 g) was dissolved in methanol and shaken with hydrogen at 35 p.s.i. over a platinum catalyst for 4 hours. The solution was filtered free of catalyst and evaporated to dryness, and the residue was crystallized from ether. The product melted at 157–158°, either alone or in admixture with the enol VIII.

Treatment of XV and VII with Hydrogen

Compound XV (0.15 g) was dissolved in methanol and shaken with hydrogen at 30 p.s.i. over a platinum catalyst for 11 hours. The solution was filtered free of catalyst and evaporated to dryness. Addition of ether gave a crystalline product which, after recrystallization from ether, melted at 206–208°. Calc. for $C_{24}H_{32}ON_2$: C, 79.1; H, 8.85; N, 7.7%. Found: C, 79.2; H, 8.85; N, 7.8%.

The infrared spectrum of XVI showed absorption in the carbonyl region in carbon tetrachloride solution at 1708 cm^{-1}. Its ultraviolet spectrum showed only non-conjugated phenyl absorption.

The same product was obtained on similar treatment of VII.

Deuterium Exchange and Deuterium Analysis

The compound (50 mg) was dissolved in deuteromethanol (CH_3OD), and 0.05 ml of

20% sodium deuteroxide was added. The reaction mixture was heated under reflux for ½ hour under an atmosphere of nitrogen and then taken to dryness. A total of four such exchanges was carried out on each sample. To the residue remaining after the last exchange there was added 0.5 ml of deuterium oxide, and the reaction mixture was extracted with ether. The ether extract was dried over anhydrous magnesium sulphate, and the ether was evaporated, leaving a residue, which was purified by sublimation or crystallization from a non-hydroxylic solvent.

The deuterated samples were analyzed for their deuterium content by the method of Jones and MacKenzie (17). Deuterobiphenyl, $C_{12}D_{10}$, was used as a standard to check the analytical method. The results are tabulated below.

Compound	Number of deuterium atoms per molecule
Biphenyl	10.1, 10.0, 10.3
Lycopodine	2.74, 2.78, 2.74
α-Cyanolycopodine, VI	2.51, 2.61
α-Cyclized compound, XXI	1.95, 1.93, 1.95
β-Cyclized compound, XXII	0.00, 0.00
Hofmann product, XXIII*	1.92, 1.89, 1.92

*In this case each exchange was carried out for a 12-hour period. Under the conditions stated above the following results were obtained: 1.69, 1.65, 1.69.

Cyclized Compound XXI

The compound was prepared by the method reported previously (4, 7).

Cyclized Compound XXII

The compound was prepared by the method reported previously (4, 7).

Preparation of XIX by Dehydration and Hydride Reduction of XII

Compound XII (1.0 g) was dissolved in xylene containing *p*-toluenesulphonic acid (0.15 g), and the mixture was refluxed for 4 hours. The reaction mixture was cooled, washed with dilute acid, dilute sodium bicarbonate, and water. On removal of the xylene under reduced pressure on the steam bath, there remained a mobile oil (0.75 g) which failed to crystallize even after chromatography on alumina.

The infrared absorption spectrum of a film of this compound showed weak absorption in the unsaturation region near 1650 cm^{-1} and lacked hydroxyl absorption. The cyanamide absorption appeared at 2210 cm^{-1}.

The anhydro compound (1.6 g), prepared as described above, was added to a solution of 1.0 g of lithium aluminum hydride in boiling ethyl ether. The mixture was refluxed in a nitrogen atmosphere overnight. The excess lithium aluminum hydride was decomposed with moist ether; water was added and the mixture extracted with chloroform. On distillation of the chloroform there remained 1.2 g of an oil. This crude material was dissolved in ether and the basic fraction extracted with dilute hydrochloric acid. The acid extract was made alkaline with ammonia and the free base removed by ether extraction. A sample of this amorphous base was dissolved in a small volume of acetone. Concentrated hydrochloric acid was added and crystals of the hydrochloride formed. Recrystallization from acetone produced colorless needles, m.p. 245–247° C. Calc. for $C_{16}H_{27}N.HCl$: C, 71.2; H, 10.10; N, 5.2%. Found: C, 71.5; H, 10.20; N, 5.0%.

In carbon tetrachloride, the free base XIX showed infrared absorption in the unsaturation region at 1655 cm^{-1} and lacked cyanamide absorption. The hydrochloride showed a strong band at 1585 cm^{-1} in nujol or in chloroform, attributed to the ionized salt of the secondary amine.

Dehydrogenation of XIX

The dehydrogenation was carried out in a 15-ml, round-bottomed flask sealed to a 15-cm tube which served as an air condenser for the refluxing mixture. The apparatus was constructed so that it could be evacuated, flushed with nitrogen, and the reaction carried out in a slow stream of purified nitrogen. A small thermometer was inserted into the reaction flask to permit direct reading of the temperature of the mixture.

Compound XIX (0.80 g) was mixed with 0.80 g of selenium powder in the dehydrogenation apparatus. The apparatus was flushed with nitrogen several times and then inserted into an electrically heated salt bath maintained initially at 250°. The temperature of the reaction mixture was raised to 295° in ½ hour, at which time the decomposition appeared to be rapid. The dehydrogenation was allowed to continue overnight in a slow stream of nitrogen and at a temperature up to 300°. The residue was removed from the apparatus with boiling chloroform. The chloroform extract was evaporated to dryness and the residue treated with boiling ethyl ether. The ether-soluble material was extracted repeatedly with dilute hydrochloric acid, and the combined acid extract was basified and then extracted repeatedly with ether. The crude base isolated in this manner amounted to 0.37 g. This material was dissolved in a small volume of cyclohexane and chromatographed on alumina. The progress of the fractionation was followed by recording the ultraviolet spectra of the eluates. The first eluates showed identical spectra. These were combined, and the solvent was evaporated to yield 0.07 g of a clear oil. This material was dissolved in a small volume of ether and added to a solution of an equal weight of picric acid dissolved in refluxing ether. There was immediate separation of glistening, yellow plates which, after recrystallization from ether, melted at 160–162°. Calc. for $C_{22}H_{24}N_4O_7$: C, 58.0; H, 5.26; N, 12.3%. Found: C, 58.3; H, 5.27; N, 12.2%.

The free base recovered from the picrate was analyzed on a high-temperature mass spectrometer and found to have a parent mass of 227, corresponding to $C_{16}H_{21}N$. The ultraviolet spectrum of this compound was typical of alkyl quinolines and was remarkably similar to that of 7,8-dimethylquinoline.

Preparation of Cyclized Product XXIII

Lycopodine was dissolved in acetone, and methyl iodide added. The methiodide separated immediately and virtually quantitatively and was recrystallized from methanol–acetone.

Lycopodine methiodide (3.3 g) was added to a solution of potassium (1.5 g) in *t*-butyl alcohol (100 ml). The mixture was heated under reflux for 12 hours and then evaporated to dryness. Water was added to the residue and the aqueous mixture extracted with ether. The dried ether extract was evaporated to dryness and dissolved in acetone, and the base was precipitated as its hydrochloride by addition of concentrated hydrochloric acid. The hydrochloride was dissolved in water, and the solution was made basic with ammonia and then extracted with ether. Evaporation of the ether gave a crystalline residue which was recrystallized from petroleum ether to yield compound XXIII which melted at 82°. The crude yield was 86%. Calc. for $C_{17}H_{27}ON$: C, 78.2; H, 10.34; N, 5.4%. Found: C, 78.1; H, 10.21; N, 5.3%.

The compound showed a strong band at 1695 cm^{-1} in its infrared spectrum. There were no bands which might be attributed to an olefin or olefinic hydrogens. The N.M.R. spectrum showed the presence of $\diagup\!\!\diagdown N—CH_3$ and $\diagup\!\!\diagdown CHCH_3$ groups.

Treatment of XXIII with Cyanogen Bromide

A small sample of XXIII was dissolved in dry benzene and treated with an excess of cyanogen bromide in dry benzene, and the mixture was allowed to stand overnight in the refrigerator. The cyanogen bromide and benzene were removed under reduced pressure, and the residue was taken up in chloroform, washed with acid, alkali, and water, dried, and taken to dryness. The residue formed a crystalline solid which was recrystallized from ether and melted at 162° C. Calc. for $C_{17}H_{24}ON_2$: C, 75.0; H, 8.82; N, 10.3%. Found: C, 74.9; H, 8.71; N, 10.3%.

ACKNOWLEDGMENTS

The authors wish to express their thanks to the National Research Council of Canada, and to the Ontario Research Foundation, for financial assistance.

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LYCOPODIUM ALKALOIDS

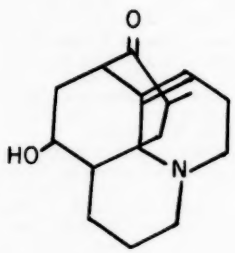
XI. THE STRUCTURE AND REACTIONS OF ACRIFOLINE[1]

W. N. FRENCH[2] AND D. B. MACLEAN

ABSTRACT

Structure A for acrifoline is proposed from a study of the products formed in the Hofmann degradation, von Braun degradation, and several oxidative degradations of the alkaloid. Structure C is proposed for a compound formed on treatment of dihydroacrifoline with copper sulphate in pyridine or with peroxytrifluoroacetic acid.

In a preliminary communication (1) we reported that acrifoline, $C_{16}H_{23}O_2N$, a minor alkaloid of *Lycopodium annotinum*, (2) had structure A. This structure has received support from the work of Anet (3), who has succeeded in interrelating acrifoline and several other *Lycopodium* alkaloids.



A

We now wish to report in greater detail the evidence in favor of this structure. Previously, Perry and MacLean (4) had established that acrifoline had a double bond, a hydroxyl group, a carbonyl group, and a tertiary nitrogen atom, and therefore was tetracyclic. The application of nuclear magnetic resonance spectroscopy has provided further information on the nature of these functional groups.

The N.M.R. spectrum of acrifoline showed absorptions of area 1 at displacements of 3.35 and 2.04 p.p.m.* attributed to $\diagdown\!\!\diagup$CHO— and $\diagdown\!\!\diagup$C=C$\diagup^{H}$ groups respectively. A doublet of area 3, occurring at a displacement of 6.20 p.p.m., established the presence of a $\diagdown\!\!\diagup$CHCH$_3$ group. The presence of one C-methyl group was also shown by Kuhn–Roth analysis of acrifoline (as its hydrobromide salt). An examination of the N.M.R. spectrum of acetylacrifoline (4) confirmed the above results. Its N.M.R. spectrum

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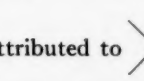
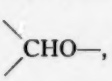
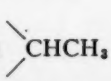
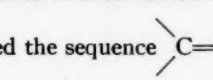
Contribution from the Department of Chemistry, McMaster University, Hamilton, Ontario.
[2]*Holder of a National Research Council Studentship, 1958–60.*
**Chemical shifts are given in parts per million (p.p.m.) from chloroform.*

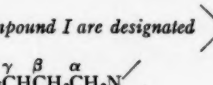
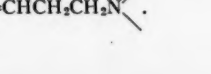
showed absorptions attributed to  ,  , and  at displacements of 1.97, 2.64, and 6.30 p.p.m. respectively. A sharp peak at a displacement of 5.50 p.p.m. was assigned to the $—COCH_3$ group. The absence of absorption at low field in both of the above N.M.R. spectra rules out the presence of an aldehyde group and therefore, the carbonyl group of acrifoline must be ketonic. The hydroxyl group is secondary since the signal area of the CHO group corresponded to one proton.

A clue to the spatial relationship of the carbonyl and hydroxyl groups came from infrared spectroscopy. The infrared spectrum of acrifoline in nujol showed hydroxyl absorption at 3310 cm^{-1}, but no carbonyl absorption, while in solution it exhibited strong carbonyl absorption at 1700 cm^{-1}, as well as the usual hydroxyl absorption. Both spectra showed ether absorption near 1100 cm^{-1}. The absence of carbonyl absorption in the solid state implies that acrifoline crystallizes as a hemiketal, whereas the presence of carbonyl absorption in solution indicates that an equilibrium exists between the hemiketal and the hydroxy-ketone forms. Since only five- and six-membered cyclic hemiketals form spontaneously, the carbonyl group and the carbon atom bearing the hydroxyl group must be separated by two or three atoms. The same phenomenon was also observed in many derivatives of acrifoline which contained the original carbonyl and hydroxyl groups.

A study of the products formed in the Hofmann degradation, in the von Braun degradation, and in several oxidative degradations led to the proposal of structure A for acrifoline. Each of these degradative approaches will be discussed in detail below.

The Hofmann degradation of acrifoline established the sequence  in the molecule. Acrifoline readily formed a methiodide which, on treatment with potassium tertiary butoxide in boiling tertiary butanol, underwent Hofmann elimination to give a crystalline base (I), $C_{17}H_{25}O_2N$, in 70% yield. The infrared spectrum of I showed strong absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}, characteristic of a terminal methylene group. Strong absorption in the ultraviolet spectrum with a maximum at 2400 Å (ϵ = 24,000), along with an enhanced double-bond absorption in the infrared at 1625 cm^{-1}, showed that a conjugated diene system was present in compound I, and that the double bond introduced during the Hofmann reaction was in conjugation with the original double bond of acrifoline. Barring rearrangement, the original double bond in acrifoline must have occupied a γ,Δ position* relative to nitrogen. Ozonolysis of compound I, and subsequent isolation and identification of formaldehyde, confirmed the presence of a $=CH_2$ group. No other volatile carbonyl compound was isolated from the ozonolysis, nor were any non-volatile fragments characterized.

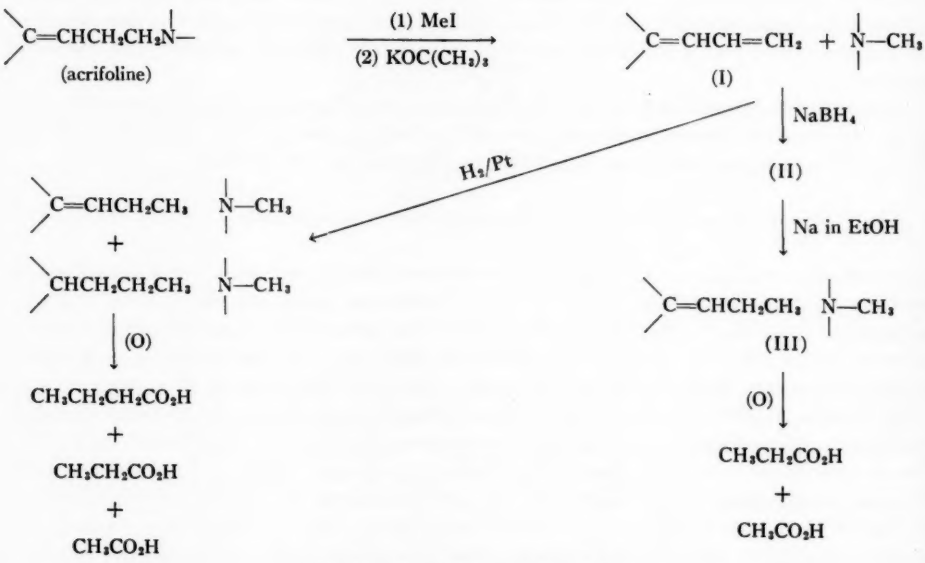
Catalytic reduction of the diene system in compound I led to the formation of a non-crystalline product which was probably a mixture of partially reduced and fully reduced compounds. A quantitative microhydrogenation of compound I showed the rapid uptake of one mole of hydrogen while the second mole was only partially consumed after several hours. However, chemical reduction of the diene system resulted in the formation of a

*The four carbon atoms in the conjugated diene system in compound I are designated  to facilitate the present discussion. Acrifoline is designated  .

1,2-reduced product. The carbonyl group in compound I was first reduced with sodium borohydride to give a diol (II), $C_{17}H_{27}O_2N$. Treatment of II with sodium in alcohol gave III, $C_{17}H_{29}O_2N$. Compound III was oxidized by a modified Kuhn–Roth method (5) and yielded acetic acid and propionic acid. The acids were identified by vapor-phase chromatography of their methyl esters. The isolation of propionic acid established the presence of an ethyl group in III and proved that a 1,2-reduction had occurred with sodium in alcohol. Acrifoline was subjected to the same treatment and yielded only acetic acid, as expected.

Modified Kuhn–Roth oxidation of the crude material obtained by hydrogenation of the Hofmann product, I, yielded butyric acid as well as propionic and acetic acids. Therefore, a *n*-propyl group was present in the product of complete hydrogenation of compound I.

The N.M.R. spectrum of acrifoline established that the double bond was trialkylated. The oxidation studies show that the double bond must occupy a γ,Δ-position relative to nitrogen with the Δ C-atom dialkylated. The preceding series of reactions is summarized in the chart below.



Products from Hofmann degradation

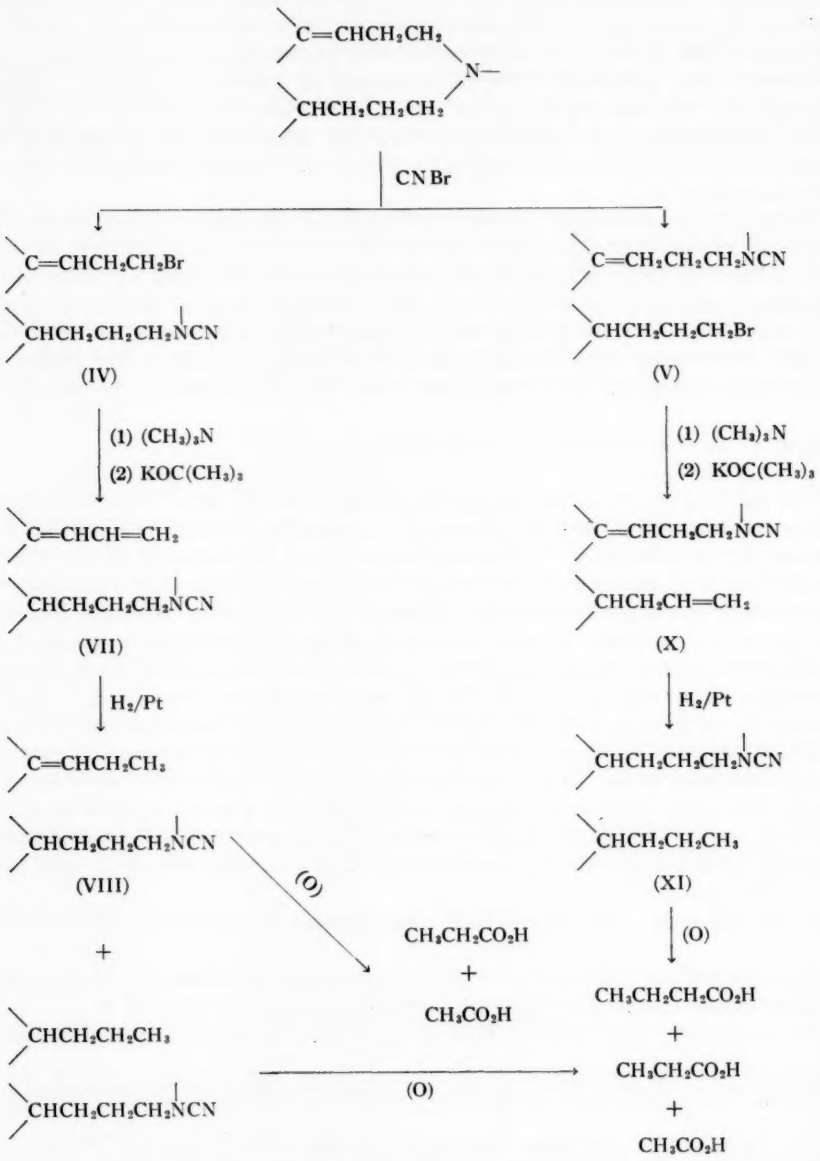
A study of the products of the von Braun reaction of acrifoline confirmed the results of the Hofmann degradation and, in addition, allowed an extension of the peripheral structure to $-CH_2CH_2CH_2-\underset{|}{N}-CH_2CH_2CH=C\diagup$. Reaction of acrifoline with cyanogen bromide yielded two isomeric cleavage products, α- and β-cyanobromoacrifoline, IV and V, respectively. β-Cyanobromoacrifoline (V), $C_{17}H_{23}O_2N_2Br$, was isolated in 30% yield as a crystalline product and α-cyanobromoacrifoline (IV) was obtained in

22% yield as a crystalline quaternary ammonium bromide (VI), $C_{20}H_{32}O_2N_3Br$, on treatment of the mother liquor from the isolation of β-cyanobromoacrifoline (V) with trimethylamine. The quaternary ammonium bromide VI readily eliminated trimethylamine on treatment with potassium tertiary butoxide and formed a neutral product (VII), $C_{17}H_{22}O_2N_2$. The presence of a diene system in VII similar to that in I was established by an examination of its infrared and ultraviolet spectrum. One can conclude that the same ring cleavage occurred in the formation of both Hofmann product I, and α-cyanobromoacrifoline (IV).

Treatment of compound VII with hydrogen and platinum resulted mainly in a 1,2-reduction of the conjugated diene system. The crystalline product VIII, $C_{17}H_{24}O_2N_2$, was isolated in 50% yield, and gave propionic and acetic acids on oxidation by the modified Kuhn–Roth procedure. Some fully saturated material containing a *n*-propyl side chain was also formed along with compound VIII since oxidation of the material in the mother liquor from the isolation of VIII yielded a small amount of butyric acid in addition to propionic and acetic acids. These results substantiate the presence of the sequence $\diagdown C{=}CHCH_2CH_2N \diagup$, in acrifoline.

The reaction of crystalline β-cyanobromoacrifoline (V) with trimethylamine gave a non-crystalline, ether-insoluble quaternary ammonium bromide (IX). Other reactions carried out on compound V, including reduction of the carbonyl group with sodium borohydride and removal of the bromine atom with hydrogen and palladium at room temperature or dry-ice temperature, also gave non-crystalline products. Treatment of the quaternary bromide IX with potassium tertiary butoxide gave an oil (X) which contained a terminal methylene group, as indicated by its infrared spectrum, and confirmed by the formation of formaldehyde upon ozonolysis. The terminal double bond was not in conjugation with the original double bond since there was no intense absorption in the ultraviolet region. Hydrogenation of the diolefin X gave an oil that partially crystallized from ether. This crystalline product (XI), $C_{17}H_{26}O_2N_2$, gave butyric, propionic, and acetic acids in an over-all yield of 68%, in a molar ratio of approximately 44:14:42, on modified Kuhn–Roth oxidation. The presence of butyric acid among the oxidation products proved that compound XI had a *n*-propyl side chain, and therefore, that the sequence $-\overset{|}{\underset{|}{C}}CH_2CH_2CH_2N \diagup$ was present in acrifoline. The relatively high yield of butyric acid indicated that the *n*-propyl group in compound XI was not joined to a quaternary center. The reactions are summarized in the chart below.

The sequence $-COCH(CH_3)CH_2-$ in acrifoline, as well as the relationship of this sequence to the $\diagdown CHOH$ function, was established by a study of the products of selenium dioxide oxidation of acrifoline. The major product XII, $C_{16}H_{21}O_2N$, of this reaction proved to be an α,β-unsaturated ketone. The infrared spectrum of its perchlorate showed carbonyl absorption at 1690 cm^{-1} and enhanced double-bond absorption at 1627 cm^{-1}, and the ultraviolet spectrum of its perchlorate exhibited a maximum at 2430 Å ($\epsilon = 5330$). The possibility of skeletal rearrangement during the formation of compound XII was excluded by hydrogenation of compound XII to dihydroacrifoline.



Products from von Braun degradation

The N.M.R. spectrum of compound XII had two peaks of area 1 at displacements of 1.96 and 3.37 p.p.m. representing the isolated double-bond proton and the $\diagdown$CHO

group respectively. A single, sharp peak at a displacement of 5.50 p.p.m. indicated that the methyl group was located on the double bond α,β to the carbonyl group. A single peak of area 1 occurring at a displacement of 0.23 p.p.m. was attributed to a proton on the β-carbon of the α,β-unsaturated ketone.

The minor product, XIII, isolated from the reaction of acrifoline with selenium dioxide had the same molecular formula, $C_{16}H_{21}O_2N$, as the major product XII. However, the ultraviolet spectrum of this material (as the perchlorate) showed no strong absorption while its infrared spectrum showed carbonyl absorption at 1725 cm^{-1}, but no hydroxyl absorption. Conversion of XII to XIII by treatment with base indicated that XIII was formed in the reaction medium by intramolecular addition of the hydroxyl group to the conjugated double-bond system. The ease of this addition implies that a five- or six-membered cyclic ether is formed, and that the carbon atom β to the carbonyl group and the carbon atom carrying the hydroxyl group are separated by two or three atoms. The N.M.R. spectrum of compound XIII had a doublet at a displacement of 6.22 p.p.m. representing the $\diagdown\!\!\diagup CHCH_3$ group, and a peak of area 1 at a displacement of 1.99 p.p.m. representing the single proton on the double bond. Two peaks, each of area 1, occurring at displacements of 3.19 and 3.54 p.p.m., were attributed to absorption by the two CHO groups, one group at either end of the ether linkage. The N.M.R. results on XII and XIII can only be explained if the sequence $-COC(CH_3)=CH-$ is present in the former and the sequence $-COCH(O)CH(CH_3)-$ in the latter.

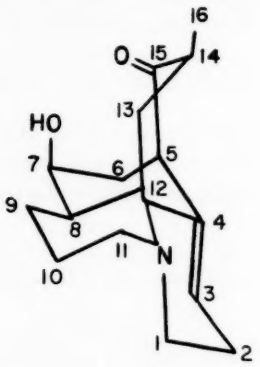
Oppenauer oxidation of dihydroacrifolinol* with aluminum tertiary butoxide and cyclohexanone in boiling toluene gave a crude product that showed two strong absorptions of equal magnitude at 1705 and 1735 cm^{-1} in the carbonyl region of the infrared spectrum, and hydroxyl absorption at 3500 cm^{-1}. Chromatography of the crude product led to the isolation of only one compound (XIV), $C_{16}H_{23}O_2N$, in 45% yield. The infrared spectrum of XIV showed a single, strong carbonyl absorption at 1705 cm^{-1} and no hydroxyl absorption. Compound XIV is probably a diketone with both carbonyl groups located in rings that are six-membered or larger. Absorption at 1420 cm^{-1} in the spectra of both the base XIV and its hydrobromide and none in this region in the spectrum of acrifoline (nujol, chloroform, or carbon tetrachloride) implies that a $-CH_2CO-$ group is present in compound XIV but is absent in acrifoline (6). Thus the $-CH_2CO-$ group in the diketone XIV must have arisen from a $-CH_2CHOH-$ group in acrifoline.

The occurrence of peaks at 1735 cm^{-1} and 3500 cm^{-1} in the infrared spectrum of crude compound XIV may be due to the presence of an aldol impurity containing a five-membered ketone ring.

**Previously, two dihydroacrifolinols were reported (4). α-Dihydroacrifolinol was reported to form by hydrogenation of acrifoline to dihydroacrifoline followed by reduction of the carbonyl group with lithium aluminum hydride. β-Dihydroacrifolinol was formed by initial reduction of the carbonyl group in acrifoline to give acrifolinol followed by hydrogenation. In the present investigation, the same product, melting at 192.5–193.0° and identical with α-dihydroacrifolinol, was formed by either of the above procedures.*

Structure A, which has been formulated for acrifoline, is in accord with the data presented above. It explains the existence of the hemiketal formed by interaction between the carbonyl and the hydroxyl functions. The facile conversion of the unsaturated compound XII to its saturated isomer XIII is readily accommodated by this structure. The separation of the double bond and the carbonyl group by a bridgehead carbon explains why an interaction between these two groups was never observed in acrifoline or its degradation products. The structure is biogenetically feasible and is compatible with the proposal recently put forward by Conroy (7). Furthermore, this structure is corroborated by the work, on annofoline, of Anet (8), who has also shown that annofoline is the C_4 epimer of dihydroacrifoline (4).

The stereochemistry of acrifoline has been discussed by Anet (3), who has proposed that it has the configuration shown in the perspective formula B below.



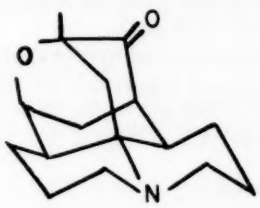
B

The only center where configuration might be in doubt is C_8, and the assignment shown is supported by the following experiments. Acrifoline underwent Wolff–Kishner reduction to XV, $C_{16}H_{25}ON$. The latter was converted by chromic acid oxidation to the ketone (XVI), $C_{16}H_{23}ON$, which was stable to the action of alkali.

Treatment of dihydroacrifoline, with copper sulphate in pyridine or with peroxytrifluoroacetic acid, resulted in the formation of a new compound (XVII), $C_{16}H_{23}O_2N$. The structure of this compound has been established and its formation involved an unexpected ether-bridging reaction.

Product XVII was formed in 78% yield on treatment of dihydroacrifoline with copper sulphate in pyridine. In its infrared spectrum there was a peak at 1725 cm^{-1} attributed to a carbonyl group, but no absorption which could be attributed to a hydroxyl group. The carbonyl peak was shifted by 25 cm^{-1} from its position in the starting material. The N.M.R. spectrum of XVII had absorption of area 1 at a displacement of 3.55 p.p.m. due to a $\diagdown$CHO— group, and a sharp, single peak at a displacement of 6.16 p.p.m. due to the methyl group on a carbon atom not carrying a proton. Compound XVII underwent reduction to a secondary alcohol (XVIII) with sodium borohydride and reacted with phenyllithium to form a phenylcarbinol (XIX). The alcohol XVIII yielded a monoacetate (XX) on treatment with a mixture of acetic acid and trifluoroacetic anhydride

and was converted with chromic acid to the ketone XVII. Compound XVII was, therefore, assigned structure C.



C

The over-all reaction closely resembles the oxidation of other aldehydes and ketones by various reagents (9, 10), in which oxidation occurs by a free radical process at the α-carbon atom after enolization has occurred.

The same product was obtained on attempted Baeyer–Villiger oxidation of dihydroacrifoline. The reaction was carried out under standard conditions (11, 12) using peroxytrifluoroacetic acid in methylene chloride in the presence of disodium phosphate.

EXPERIMENTAL

Acrifoline

Acrifoline was obtained from annotoxine according to the procedure of Perry and MacLean (4). Calc. for $C_{16}H_{23}O_2N.HBr$: one C-methyl, 4.39%. Found: C-methyl, 4.15%.

Acetyl Acrifoline

Acetyl acrifoline was prepared from acrifoline by the procedure of Perry and MacLean (4).

Preparation of Acrifoline Methiodide

A solution of acrifoline (1.0 g) in 15 ml of acetone and 3 ml of methyl iodide was heated under reflux and, within 2 minutes, a crystalline methiodide began to separate. The mixture was heated for 15 minutes longer, cooled, and allowed to stand at room temperature for 1 hour. Filtration gave 1.23 g (80%) of product melting at 278–279° (with decomposition). An additional 0.260 g (17%) of product was obtained from the filtrate on further treatment with methyl iodide in acetone. Recrystallization of the methiodide from methanol–acetone gave colorless needles melting at 280–281° (with darkening). Bertho and Stoll (13) report a melting point of 249–250°, and Achmatowicz and Rodewald (14), a melting point of 267–268° for this compound. Calc. for $C_{16}H_{23}O_2N.CH_3I$: C, 50.6; H, 6.49; N, 3.47%. Found: C, 50.5; H, 6.47; N, 3.12%.

Treatment of Acrifoline Methiodide with Potassium Tertiary Butoxide

A mixture of acrifoline methiodide (1.10 g) and potassium tertiary butoxide (1.50 g) in 60 ml of tertiary butanol and 2 ml of benzene was heated under reflux for 4 hours. The mixture was cooled, water was added, and the organic solvents were removed under reduced pressure. The resulting alkaline aqueous mixture was extracted several times

with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual oil was crystallized from petroleum ether to give 0.53 g (70%) of colorless needles melting at 158–159°. Recrystallization from petroleum ether gave compound I which melted sharply at 161°. Calc. for $C_{17}H_{25}O_2N$: C, 74.1; H, 9.15%. Found: C, 73.7; H, 9.02%.

The infrared spectrum of this material had hydroxyl absorption at 3500 cm^{-1}, carbonyl absorption at 1700 cm^{-1}, double-bond absorption at 1650 cm^{-1} (weak) and 1625 cm^{-1} (strong), and $=CH_2$ absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The ultraviolet spectrum had $\lambda_{max}^{C_2H_5OH}$ at 2400 Å, $\epsilon = 24{,}000$.

Ozonolysis of the Hofmann Product I

A stream of ozone-enriched oxygen was passed through a solution of the Hofmann product (0.10 g) in 15 ml of glacial acetic acid until the uptake of ozone was complete (about 20 minutes), then zinc dust (0.50 g) was added and the mixture steam distilled.

Formaldehyde was isolated from the steam distillate as its 2,4-dinitrophenylhydrazone and as its dimedone derivative. The melting points of both derivatives agreed with recorded values, and showed no depression on admixture with authentic samples.

Reduction of I with Sodium Borohydride

A solution of compound I (0.20 g) and $NaBH_4$ (0.2 g) in 15 ml of absolute ethanol was allowed to stand at room temperature for 6 hours. The excess borohydride was destroyed with formaldehyde, water was added, and the organic solvent was removed under reduced pressure. The product was isolated by extraction of the aqueous alkaline mixture with chloroform, extraction of this chloroform solution with dilute aqueous acid, and extraction of the basified aqueous solution with chloroform. The latter chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure, yielding 0.20 g of crystalline solid melting at 249–251°. An analytical sample of compound II, m.p. 251.5–252.0°, was obtained after two recrystallizations from methanol–acetone. Calc. for $C_{17}H_{27}O_2N$: C, 73.6; H, 9.81; N, 5.05; NCH_3, 10.5%. Found: C, 73.4; H, 9.83; N, 5.07; NCH_3, 10.8%.

The infrared spectrum had hydroxyl absorption in the 3000–3200 cm^{-1} region, no carbonyl absorption, double-bond absorption at 1625 and 1590 cm^{-1}, and $=CH_2$ absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The ultraviolet spectrum had $\lambda_{max}^{C_2H_5OH}$ at 2400 Å, $\epsilon = 28{,}000$.

Sodium in Alcohol Reduction of the Diol II

A solution of compound II (0.30 g) in 75 ml of absolute ethanol was heated to boiling, and sodium was added in small pieces over a period of 3½ hours at such a rate that metallic sodium was always present in the medium. The solution was cooled, ethanol and water were added, and the organic solvent was removed under reduced pressure. The resulting aqueous mixture was extracted several times with chloroform. The combined, pale yellow chloroform solution was extracted with dilute aqueous acid and the acid extract made alkaline with ammonia and extracted with chloroform. The latter chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual colorless oil was crystallized from acetone–ether, yielding 0.264 g (88%) of compound III which melted at 191.5–192.5°. Recrystallization from acetone–ether raised the melting point to 194.5–195.0°. Calc. for $C_{17}H_{29}O_2N$: C, 73.0; H, 10.5%. Found: C, 72.8; H, 10.5%.

Neither the infrared nor ultraviolet spectrum of this compound showed absorption characteristic of a conjugated diene system.

Oxidative Determination of Alkyl Side Chains

The oxidations were carried out and the acids analyzed by the method described by Harrison (5).

Modified Kuhn–Roth Oxidation of Acrifoline

Oxidation of 0.025 g (0.096 millimole) of acrifoline yielded 0.082 milliequivalent (86%) of volatile carboxylic acid. Vapor-phase chromatography of its methyl ester indicated only the presence of methyl acetate.

Modified Kuhn–Roth Oxidation of the 1,2-Reduced Hofmann Product III

Oxidation of 0.030 g (0.108 millimole) of compound III yielded 0.183 milliequivalent (85%) of volatile carboxylic acids. Vapor-phase chromatography of their methyl esters revealed the presence of methyl acetate and methyl propionate in the molar ratio of 67:33 respectively.

Modified Kuhn–Roth Oxidation of Crude Hydrogenated Hofmann Product

The Hofmann product I (0.030 g, 0.109 millimole) was dissolved in methanol and treated with hydrogen (50 p.s.i.g.) and platinum (0.025 g PtO_2) for 15 hours. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure, leaving a colorless oil. Chromic acid oxidation of this crude product yielded 0.218 milliequivalent of volatile carboxylic acids. Vapor-phase chromatography of their methyl esters revealed the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratio of 77:19:4 respectively.

Reaction of Acrifoline with Cyanogen Bromide

(a) Isolation of β-Cyanobromoacrifoline V

A solution of acrifoline (1.0 g) and cyanogen bromide (1.0 g) in 15 ml of anhydrous benzene was allowed to stand at room temperature for 40 hours. The solvent and excess cyanogen bromide were removed under reduced pressure and the residue taken up in chloroform, washed with dilute acid, water, and aqueous sodium bicarbonate. Acrifoline (0.13 g) was recovered from the acid extract. The chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving 1.0 g of a pale yellow oil, which was taken up in acetone–ether and placed in the refrigerator. After several hours, 0.41 g (30%) of colorless crystals, melting at 153–154°, were removed by filtration. Recrystallization from acetone–ether raised the melting point to 154.5°. Calc. for $C_{17}H_{23}O_2N_2Br$: C, 55.6; H, 6.31; N, 7.63%. Found: C, 56.0; H, 6.67; N, 7.92%.

The infrared spectrum of this material in nujol showed cyanamide absorption at 2200 cm^{-1} and hydroxyl absorption at 3300 cm^{-1}, but no absorption in the carbonyl region. The infrared spectrum in chloroform showed hydroxyl absorption at 3580 cm^{-1}, cyanamide absorption at 2205 cm^{-1}, weak carbonyl absorption at 1710 cm^{-1}, and double-bond absorption at 1670 cm^{-1}.

(b) Isolation of α-Cyanobromoacrifoline IV as its Quaternary Trimethylammonium Bromide VI

The filtrate from the separation of the crystalline bromide V was evaporated to dryness, yielding an oil, which was dissolved in 5 ml of absolute ethanol containing trimethylamine (0.00075 equiv. per ml). After the solution had remained for 24 hours at room temperature, the solvent and excess trimethylamine were removed under reduced pressure. The residue

partially crystallized on addition of acetone, and on filtration yielded 0.364 g (22%, based on the weight of acrifoline used) of colorless crystals melting at 276.0–276.5°. Recrystallization from methanol–acetone raised the melting point to 276.5–277.0°. Calc. for $C_{20}H_{22}O_2N_3Br$: C, 56.3; H, 7.57; N, 9.86%. Found: C, 56.5; H, 7.57; N, 9.66%.

The infrared spectrum showed hydroxyl absorption at 3300 cm^{-1}, cyanamide absorption at 2210 cm^{-1}, double-bond absorption at 1655 cm^{-1}, and weak carbonyl absorption at 1700 cm^{-1}.

Treatment of VI with Potassium Tertiary Butoxide

A mixture of the quaternary ammonium bromide VI (0.250 g) and potassium tertiary butoxide (0.50 g) in 25 ml of tertiary butanol and 1 ml of benzene was heated under reflux for 2 hours. The mixture was cooled, water was added, and the organic solvent was removed under reduced pressure. The resulting alkaline aqueous mixture was extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, yielding a colorless oil which crystallized from petroleum ether. The first crop of compound VII weighed 0.135 g (80%) and melted at 183.0–183.5°. Recrystallization from ether – petroleum ether did not raise the melting point. Calc. for $C_{17}H_{22}O_2N_2$: C, 71.3; H, 7.75; N, 9.78%. Found: C, 71.4; H, 7.67; N, 9.86%.

The infrared spectrum in nujol showed terminal methylene absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}, double bond absorption at 1640 cm^{-1} and 1590 cm^{-1}, cyanamide absorption at 2210 cm^{-1}, and hydroxyl absorption at 3300 cm^{-1}. The infrared spectrum in chloroform showed carbonyl absorption at 1705 cm^{-1} and hydroxyl absorption at 3480 cm^{-1} as well as the absorptions due to unsaturation and the cyanamide group. The ultraviolet spectrum had $\lambda_{max}^{95\%\ EtOH}$ at 2400 Å, $\epsilon = 24{,}800$.

Hydrogenation of VII

Compound VII (0.087 g) was dissolved in 20 ml of methanol and treated with hydrogen (50 p.s.i.g.) and platinum (0.050 g PtO_2) for 15 hours. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The colorless residue was dissolved in chloroform, and the chloroform solution was washed first with dilute hydrochloric acid and then with aqueous sodium bicarbonate, and dried over anhydrous sodium sulphate. Evaporation of the chloroform under reduced pressure left a colorless oil which partially crystallized from ether. A total of 0.045 g (50%) of crystalline product VIII, melting at 154–155°, was obtained. This material was sublimed at 0.001 mm and 140° for analysis. Calc. for $C_{17}H_{24}O_2N_2$: C, 70.8; H, 8.39%. Found: C, 70.3; H, 8.37%.

The infrared spectrum of this compound had broad hydroxyl absorption in the 3400 cm^{-1} region, weak carbonyl absorption at 1710 cm^{-1}, and cyanamide absorption at 2240 cm^{-1}. In chloroform solution, the infrared spectrum of VIII showed hydroxyl absorption at 3650 cm^{-1}, weak carbonyl absorption at 1710 cm^{-1}, cyanamide absorption at 2240 cm^{-1}, and strong ether absorption at 1100 cm^{-1}.

Modified Kuhn–Roth Oxidation of VIII

Oxidation of 0.0134 g (0.047 millimole) of VIII yielded 0.068 milliequivalent (73%) of volatile carboxylic acids. Vapor-phase chromatography of their methyl esters indicated the presence of methyl acetate and methyl propionate in the molar ratio of 69:31 respectively.

Modified Kuhn–Roth Oxidation of Crude Hydrogenated α-Cyano Compound VII

The filtrate obtained from the isolation of crystalline VIII was taken to dryness and

the residual oil distilled at 0.001 mm and 130–140°. Chromic acid oxidation of a portion of this material (0.012 g, 0.045 millimole) yielded 0.061 milliequivalent (68%) of volatile carboxylic acids. Vapor-phase chromatography of their methyl esters indicated the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratios of 77:21:2 respectively.

Removal of Hydrogen Bromide from β-Cyanobromoacrifoline V

A solution of β-cyanobromoacrifoline (V) (0.20 g) in 7 ml of absolute ethanol containing trimethylamine (0.00075 equiv. per ml) was allowed to stand for 40 hours at room temperature. The solvent and excess trimethylamine were removed under reduced pressure, leaving an oil (IX) which could not be induced to crystallize.

A solution of the non-crystalline quaternary ammonium bromide IX in 20 ml of tertiary butanol and 1 ml of benzene was heated under reflux with potassium tertiary butoxide (0.60 g) for 3 hours. The mixture was cooled, water was added, and the organic solvents were removed under reduced pressure. The resulting aqueous mixture was extracted several times with chloroform. The combined chloroform extract was washed with dilute aqueous acid and aqueous sodium bicarbonate, dried over anhydrous sodium sulphate, and evaporated under reduced pressure. The residual colorless oil (X) (0.090 g, 58%) could not be induced to crystallize, even after purification by chromatography on alumina or by distillation *in vacuo*.

The infrared spectrum (film) of the oil X showed carbonyl absorption at 1705 cm^{-1}, double-bond absorption at 1635 cm^{-1}, terminal double-bond absorption at 910 cm^{-1} with an overtone at 1820 cm^{-1}, hydroxyl absorption at 3490 cm^{-1}, and cyanamide absorption at 2200 cm^{-1}. The crude oil showed no absorption in the ultraviolet region of the spectrum other than weak absorption owing to the carbonyl group.

Ozonolysis of Compound X

A solution of compound X (0.048 g) in 10 ml of glacial acetic acid was treated with an excess of ozone. Zinc dust (0.30 g) was added to the solution and the mixture steam distilled. The steam distillate (30 ml) was neutralized with sodium hydroxide, made slightly acid with acetic acid, and treated with 5 ml of dimedone solution. Five hours later, 0.044 g of needles, melting at 165–175°, was removed by filtration. Recrystallization gave 0.025 g of dimedone derivative with a sharp melting point of 190°. A mixture melting point with authentic formaldehyde dimedone derivative showed no depression.

Hydrogenation of X

Compound X, prepared as above from 0.205 g of V, was dissolved in methanol and treated with hydrogen (50 p.s.i.g.) and platinum (0.050 g of PtO_2) for 10 hours. The catalyst was removed by filtration and the solvent by evaporation under reduced pressure. The residual colorless oil was taken up in ether from which colorless crystals separated after several hours. The crystalline product XI amounted to 0.039 g (24%, based on the weight of β-cyanobromoacrifoline (V) used), and melted at 155.5–157.5°. This material was sublimed at 100–130° and 0.001 mm for analysis. Calc. for $C_{17}H_{26}O_2N_2$: C, 70.3; H, 9.03; N, 9.65%. Found: C, 70.4; H, 8.91; N, 9.43%.

The infrared spectrum of XI had a sharp absorption at 3370 cm^{-1} due to hydroxyl, strong absorption at 2200 cm^{-1} due to the cyanamide group, but no absorption in the 1600–1800 cm^{-1} region.

Modified Kuhn–Roth Oxidation of XI

Oxidation of 0.019 g (0.067 millimole) of dihydro-β-cyanoacrifoline (XI) yielded

0.086 milliequivalent (66%) of volatile carboxylic acids. Vapor-phase chromatography of their methyl esters revealed the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratios of 42:14:44 respectively.

Selenium Dioxide Oxidation of Acrifoline

A stirred solution of 0.50 g of selenium dioxide and 0.50 g of acrifoline in 25 ml of aqueous dioxane (10% water) was heated under reflux under an atmosphere of nitrogen for 8 hours. During this period, black selenium metal precipitated and the solution became deep yellow in color. The solution was cooled, diluted with 50 ml of water, and excess selenium dioxide destroyed by reduction with sulphur dioxide. The precipitated metallic selenium was removed by filtration through a sintered glass funnel and the clear, yellow acidic filtrate was washed with chloroform, made alkaline with ammonia and extracted several times with chloroform. The latter chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual yellow oil (0.415 g) was chromatographed on alumina with benzene. The first band, eluted with benzene, was a colorless oil (XIII) which formed 0.045 g (6.5%) of a crystalline perchlorate melting at 270–271° (with decomposition). Calc. for $C_{16}H_{21}O_2N.HClO_4$: C, 53.4; H, 6.16; N, 3.89%. Found: C, 53.4; H, 6.12; N, 4.05%.

The infrared spectrum of this compound had carbonyl absorption at 1725 cm^{-1}, double-bond absorption at 1680 cm^{-1}, but had no absorption in the hydroxyl region. The ultraviolet absorption spectrum showed only weak absorption at 2900 Å corresponding to a carbonyl group.

A second band was eluted from the column with chloroform. Evaporation of the solvent left a light yellow oil that partially formed a crystalline perchlorate from acetone–ether. The perchlorate (0.20 g) (30%) (m.p. 267–269°) was purified for analysis by two recrystallizations from methanol–acetone. It finally melted at 273.5–274.0°. Calc. for $C_{16}H_{21}O_2N.HClO_4$: C, 53.4; H, 6.16; N, 3.89%. Found: C, 53.6; H, 6.17; N, 3.97%.

The infrared spectrum of the perchlorate had hydroxyl absorption at 3530 cm^{-1}, carbonyl absorption at 1690 cm^{-1}, and conjugated double-bond absorption at 1627 cm^{-1}. The ultraviolet absorption spectrum had $\lambda_{max}^{C_2H_5OH}$ at 2430 Å, $\epsilon = 5330$.

The base recovered from the filtrate of the above perchlorate preparation weighed 0.150 g but was not characterized.

Conversion of the Unsaturated Compound XII to the Saturated Compound XIII

A solution of the perchlorate of the unsaturated carbonyl compound (0.020 g) in 6 ml of 95% ethanol containing sodium hydroxide (0.05 g) was allowed to stand at room temperature for 24 hours. The solution was diluted with water and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness leaving a colorless oil which formed 0.015 g (75%) of crystalline perchlorate. The infrared spectrum of this salt was identical with that of the perchlorate of the saturated carbonyl compound XIII obtained from the selenium dioxide reaction.

Oppenauer Oxidation of Dihydroacrifolinol

A solution of 0.170 g of dihydroacrifolinol (4) and aluminum tertiary butoxide (0.50 g) in 2 ml of cyclohexanone and 25 ml of toluene was heated under reflux for 7 hours, then poured into cold dilute hydrochloric acid, and the organic layer extracted several times with dilute acid. The aqueous extract was made alkaline with sodium hydroxide solution and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving a pale yellow oil whose infrared

spectrum (film) showed two intense absorptions of equal magnitude at 1705 cm^{-1} and 1735 cm^{-1} in the carbonyl region, and absorption at 3500 cm^{-1} in the hydroxyl region.

The crude base obtained above was chromatographed on alumina with 25% chloroform in benzene, and yielded a colorless oil which crystallized in poor yield from ether – petroleum ether, giving 0.016 g of solid XIV which melted at 128–129°. The infrared spectrum of this solid XIV showed a single strong carbonyl absorption at 1705 cm^{-1} and no hydroxyl absorption. A sharp peak at 1420 cm^{-1} was indicative of a $—CH_2—$ group adjacent to a carbonyl. The entire pure product obtained by chromatography was converted to the hydrobromide salt which was recrystallized twice from methanol–acetone for analysis. The total yield of salt (which melted above 315°) was 0.10 g (45%). Calc. for $C_{16}H_{23}O_2N.HBr$: C, 56.1; H, 7.07; N, 4.09%. Found: C, 56.2; H, 7.12; N, 4.11%.

The infrared spectrum of the hydrobromide showed a single, strong carbonyl absorption at 1700 cm^{-1}, but no hydroxyl absorption. A sharp peak at 1420 cm^{-1} was again indicative of a $—CH_2—$ group adjacent to a carbonyl. There was no strong absorption in the ultraviolet spectrum.

A second product of the Oppenauer oxidation containing carbonyl absorption at 1735 cm^{-1} in the infrared spectrum was not isolated.

Wolff–Kishner Reduction of Acrifoline

A solution of acrifoline (0.75 g), potassium hydroxide (2.5 g), and 95% hydrazine hydrate (2 ml) in 25 ml of ethylene glycol was heated in a flask provided with a reflux condenser and a thermometer dipping into the liquid. The solution was heated under reflux for 4 hours (reaction medium at 155°), then the apparatus was arranged for distillation. Distillation was carried out until the flask contents reached a temperature of 185°. The solution was heated under reflux for 7 hours at this temperature, then cooled, diluted with water, and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness under reduced pressure. The colorless residual oil readily crystallized and yielded 0.60 g (85%) of product which melted 122–123° on recrystallization from petroleum–ether. Calc. for $C_{16}H_{25}ON$: C, 77.7; H, 10.1; N, 5.7%. Found: C, 77.6; H, 10.3; N, 5.7%.

The infrared spectrum of this compound showed hydroxyl absorption at 3120 cm^{-1} and no carbonyl absorption. Carbonyl absorption was also absent when the spectrum was measured in chloroform.

Chromic Acid Oxidation of the Wolff–Kishner Product

A solution of 0.55 g of the Wolff–Kishner product in 20 ml of aqueous acetic acid (15% water) was cooled to −10° and treated with 1.0 g of chromic anhydride. The solution was stirred at this temperature for 1 hour, allowed to stand at 0° for 10 hours, and stirred at 0° for 4 hours. Ethanol was added to destroy excess oxidant, then water was added, and most of the acetic acid was removed by evaporation under reduced pressure. The resulting aqueous solution was made alkaline with ammonia and extracted with chloroform. The dried chloroform extract, on evaporation, yielded a colorless oil which readily crystallized without addition of solvent. Purification by sublimation at 80° and 0.001 mm gave 0.415 g (76%) of product melting at 73.5–75.0°.

The infrared spectrum showed strong carbonyl absorption at 1710 cm^{-1} and no hydroxyl absorption.

The perchlorate of this base melted at 246–247° and had strong carbonyl absorption at 1710 cm^{-1}.

Ketone XVI was recovered unchanged after treatment with sodium methoxide in methanol or potassium tertiary butoxide in tertiary butanol.

Oxidation of Dihydroacrifoline with Copper Sulphate

A solution of dihydroacrifoline (0.5 g), prepared by the method of Perry and MacLean (4), and $CuSO_4.5H_2O$ (1.2 g) in 12 ml of pyridine and 4 ml of water was heated on the steam bath for 24 hours. The deep blue solution was cooled, poured into water, and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving a yellow oil. Chromatography of this oil with chloroform on alumina yielded a colorless oil that readily formed 0.51 g (78%) of a crystalline hydrobromide (m.p. above 315°) from acetone. Calc. for $C_{16}H_{23}O_2N.HBr$: C, 56.1; H, 7.07; N, 4.09%. Found: C, 55.8; H, 6.87; N, 4.11%.

The infrared spectrum showed strong carbonyl absorption at 1720 cm^{-1} but no absorption in the hydroxyl region.

A sample of the hydrobromide of compound XVII was converted to the perchlorate salt which melted above 315°. Calc. for $C_{16}H_{23}O_2N.HClO_4$: C, 53.1; H, 6.68; N, 3.87%. Found: C, 53.4; H, 6.83; N, 3.99%.

The perchlorate showed strong absorption at 1720 cm^{-1} in the carbonyl region of the infrared spectrum, but no absorption in the hydroxyl region. Likewise, the infrared spectrum of the free base XXIV showed only carbonyl absorption and no hydroxyl absorption when measured either as a film or in chloroform solution.

Oxidation of Dihydroacrifoline with Peroxytrifluoroacetic Acid

A stock solution of peroxytrifluoroacetic acid in methylene chloride was prepared in the following manner. A solution of trifluoroacetic anhydride (6.3 ml) in 10 ml of methylene chloride was added to a cold, stirred suspension of 1 ml of 90% hydrogen peroxide in 10 ml of methylene chloride and the mixture was stirred until homogeneous and then diluted to 100 ml with methylene chloride. To 35 ml of this solution was added 0.10 g of dihydroacrifoline and 3.0 g of Na_2HPO_4. The mixture was heated under reflux for 16 hours, cooled, and poured into an aqueous solution of sulphur dioxide. After 30 minutes, the aqueous phase was made alkaline with ammonia and extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving 0.050 g of colorless oil. This oil formed hydrobromide and perchlorate salts whose infrared spectra were identical with those of the corresponding salts of the product XVII isolated from the oxidation of dihydroacrifoline with copper sulphate.

Reduction of Compound XVII

A solution of the hydrobromide of XVII (0.20 g) and sodium borohydride (0.20 g) in 25 ml of absolute ethanol was heated under reflux for 1 hour. The mixture was cooled and acetone was added to destroy excess sodium borohydride. Water was then added, and the organic solvents removed under reduced pressure. The resulting aqueous mixture was extracted with chloroform, and the dried chloroform extract evaporated under reduced pressure. Crystallization of the residual oil from ether–petroleum ether gave 0.115 g (75%) of product XVIII which melted at 182–183°. This material was sublimed at 0.001 mm and 110° for analysis. It melted at 182–183°. Calc. for $C_{16}H_{25}O_2N$: C, 73.0; H, 9.56; N, 5.32%. Found: C, 73.1; H, 9.75; N, 4.65%.

The infrared spectrum of compound XVIII showed hydroxyl absorption at 3380 cm^{-1},

no carbonyl absorption, and peaks at 1070 cm^{-1} and 1100 cm^{-1} attributed to ether absorption.

The mother liquor from the separation of the crystalline base was acidified with hydrobromic acid, and yielded 0.040 g (20%) of hydrobromide salt of compound XVIII. The hydrobromide was crystallized twice from methanol–acetone for analysis. It melted above 310°. Calc. for $C_{16}H_{25}O_2N.HBr$: C, 55.8; H, 7.61; N, 4.07%. Found: C, 56.1; H, 7.45; N, 4.45%.

The infrared spectrum showed hydroxyl absorption at 3300 cm^{-1} and ether absorption at 1075 cm^{-1} and 1105 cm^{-1}.

Preparation of the Acetate of XVIII

A solution of 0.030 g of XVIII in the mixed anhydride of trifluoroacetic acid and acetic acid (prepared by mixing 0.10 ml of glacial acetic acid and 0.16 ml of trifluoroacetic anhydride) was allowed to stand overnight at room temperature. The solution was poured into a dilute aqueous ammonia solution, and then extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving a colorless oil which slowly solidified. The solid, compound XX, was sublimed at 75° and 0.001 mm for analysis, and melted at 148–149°. Calc. for $C_{18}H_{27}O_3N$: C, 70.8; H, 8.91; N, 4.59%. Found: C, 71.1; H, 8.75; N, 4.88%.

The infrared spectrum of XX had no hydroxyl absorption but had strong acetate absorption at 1730 cm^{-1} and 1235 cm^{-1}. A strong peak at 1042 cm^{-1} was attributed to ether absorption.

Oxidation of Compound XVIII to XVII with Chromic Acid

A solution of 0.025 g of XVIII in 25 ml of 85% acetic acid–water was cooled to −15°, and treated with 0.10 g of chromic anhydride. The mixture was stirred at −15° for 2 hours, and then for 4 hours at −5°. Methanol was added to destroy excess chromic acid, and the solution allowed to stand overnight in the refrigerator. Water was added and the organic solvents removed under reduced pressure. The resulting aqueous solution was acidified with hydrochloric acid, extracted with ether, made alkaline with ammonia, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure, leaving a colorless oil. The infrared spectrum of this oil (film) was identical with that of the XVII. The oil yielded 0.028 g (86%) of hydrobromide salt whose infrared spectrum was identical with that of the hydrobromide of the XVII.

Reaction of Compound XVII with Phenyllithium

To a solution of phenyllithium in ether (prepared from 0.10 g of lithium and 1.14 g of bromobenzene in 20 ml of ether) was added 0.202 g of the copper sulphate product XVII in 15 ml of ether. The solution was heated under reflux for 2 hours, allowed to stand overnight, and poured into cold dilute hydrochloric acid. The aqueous solution was extracted several times with ether, made alkaline with ammonia, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual pale yellow oil partially solidified but a crystalline product was not obtained in good yield. The base was dissolved in acetone and treated with hydrobromic acid, yielding 0.289 g (89%) of colorless crystals melting above 315°. The salt was crystallized twice from methanol–acetone for analysis. Calc. for $C_{22}H_{29}O_2N.HBr$: C, 62.9; H, 7.19; N, 3.33%. Found: C, 62.9; H, 7.26; N, 3.32%.

The infrared spectrum of this compound had hydroxyl absorption at 3330 cm^{-1}, no

carbonyl absorption, and weak phenyl absorption at 1600 cm^{-1} and 1500 cm^{-1}. The ultraviolet spectrum showed benzenoid absorption with $\lambda_{max}^{95\%\ EtOH}$ at 2580 Å, $\epsilon = 237$.

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PARALYTIC SHELLFISH POISON

VIII. SOME CHEMICAL AND PHYSICAL PROPERTIES OF PURIFIED CLAM AND MUSSEL POISONS[1]

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ABSTRACT

Mild reduction of purified clam and mussel poisons with hydrogen yielded a nontoxic dihydro derivative. The poisons and the dihydro derivative showed no absorption in the ultraviolet but all absorbed in the infrared at 3, 6, and 9 μ. Mild oxidation of the poisons in alkaline solution produced nontoxic products with strong absorption in the ultraviolet. Preliminary studies on oxidation with permanganate or periodate and on hydrolysis with acid yielded only small fragments such as guanidopropionic acid, urea, guanidine, ammonia, and carbon dioxide. Titration of the poisons and the dihydro derivatives showed pK_a values at 8.1 and 11.5. The two poisons appear to be similar in their chemical and physical properties.

The purification of the paralytic poison found in toxic Alaska butter clams (*Saxidomus giganteus*) and in toxic mussels (*Mytilus californianus*), and some properties of these poisons, have been described in previous papers (1, 2, 3). The purified poisons[5] are white hygroscopic solids, very soluble in water and methanol, somewhat soluble in ethanol and glacial acetic acid, and insoluble in lipid solvents. They have a specific rotation of +130° and show no absorption in the ultraviolet above 220 mμ. The toxicity in white mice (Webster strain) is about 5.5×10^6 lethal doses per gram (4). The molecular formula is $C_{10}H_{17}N_7O_4 \cdot 2HCl$ (molecular weight 372), and the molecule appears to exist in two tautomeric forms. A positive color test is given by both purified poisons with the Jaffe, Benedict–Behre, and Weber reagents, but the Sakaguchi test was negative. Additional physical and chemical properties are presented here to aid in the future elucidation of the structure of the poisons.

Both clam and mussel poisons were easily reduced with hydrogen to produce dihydro derivatives which were nontoxic (2). This reduction proceeded in aqueous neutral, acid, or alkaline solutions in the presence of a platinum catalyst, and under an atmosphere of hydrogen to a maximum uptake of 1 mole of hydrogen for each mole of poison. The loss of toxicity paralleled the hydrogen uptake and when 1 mole of hydrogen was consumed, 98% of the toxicity had disappeared. This relatively simple change in chemical structure resulting in almost complete loss of toxicity has been of considerable interest in studying many properties of the poison relating to structure and toxicity. The Benedict–Behre and Jaffe tests became negative upon reduction of the poisons whereas the Sakaguchi test remained negative and the Weber test remained positive. The application of these tests to the poison and the various derivatives has been described previously (2).

The infrared spectra of the poisons and the dihydro derivative are given in Fig. 1.

[1]*Manuscript received May 8, 1961.*
Contribution from the Chemical Corps Biological Laboratories, Fort Detrick, Md.; the Chemical Laboratory of Northwestern University, Evanston, Ill.; and the Squibb Institute for Medical Research, New Brunswick, N.J. The work performed in these laboratories was carried out under contract with the Chemical Corps.
[2]*Present address: Research Laboratory, Liggett and Myers Tobacco Co., Durham, North Carolina.*
[3]*Present address: 411 Poinsettia, Lake Jackson, Texas.*
[4]*Present address: G. D. Searle and Co., P.O. Box 5110, Chicago 80, Illinois.*
[5]*Countercurrent distribution studies have indicated that the preparations described as purified poison represent a single component to the extent of 95 to 98%.*

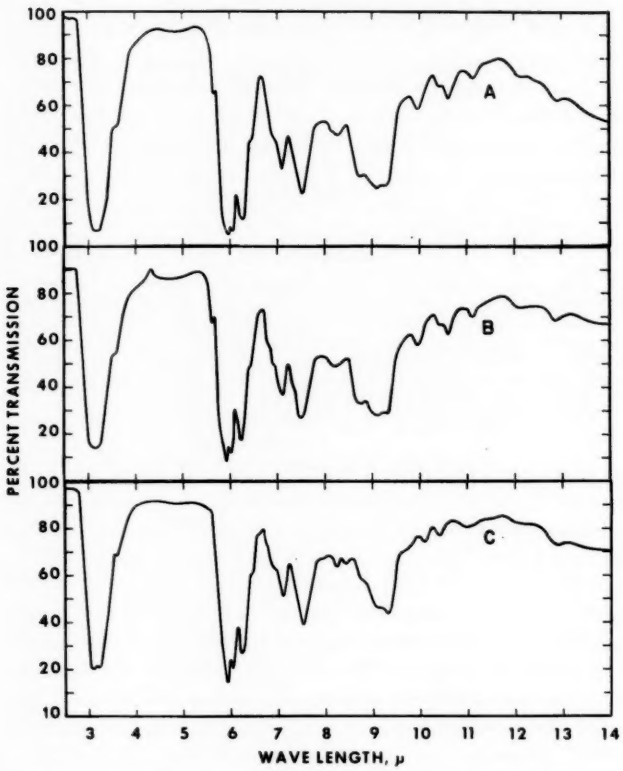

FIG. 1. Infrared spectra of (A) clam poison dihydrochloride, (B) mussel poison dihydrochloride, and (C) dihydro clam poison dihydrochloride.

The spectra for the two poisons are very similar and agree well with spectra obtained recently by Casselman *et al.* (3). The dihydro derivative showed some loss of absorption at 5.65 and 8.75 mμ.

No ultraviolet absorption above 220 mμ was observed for the poisons or for the dihydro derivatives. However, in alkaline solutions exposed to air, the poisons developed absorption maxima at 235 and 333 which reached maximum molecular extinctions of about 7000 at 235 mμ and about 5200 at 333 mμ. Under anaerobic conditions no maxima developed, which indicated that the absorption was brought about by an alkaline oxidation. Maximum extinction was reached and all toxicity disappeared when approximately 1 mole of oxygen was taken up per mole of poison (Fig. 2). The dihydro derivative did not take up oxygen in alkaline solution.

The specific rotation of both poisons (+130°) was affected by the presence of alkali, and under aerobic conditions there was a gradual decrease in the specific rotation to zero as the maximum extinctions at 235 and 333 were reached. Reduction of the poison with hydrogen did not change the rotation but eliminated the tautomeric forms.

Titration of the poisons in glacial acetic acid solution with perchloric acid and titration in aqueous solution with acid and alkali established that two base functions are present

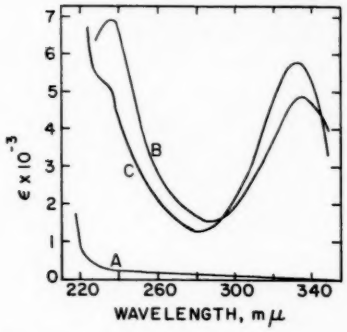

FIG. 2. Ultraviolet absorption spectra of (A) clam poison dihydrochloride, (B) alkaline oxidation product of clam poison in 0.25 *N* barium hydroxide, and (C) alkaline oxidation product of clam poison in acid solution, pH 1.

in equivalent amounts, pK_a 8.1 and ca. 11.5 (Fig. 3). No strong acidic group was found. Aqueous titration of the dihydro compounds from clam and mussel poisons gave results similar to those described for the poisons (pK_a 8.3 and ca. 11.5).

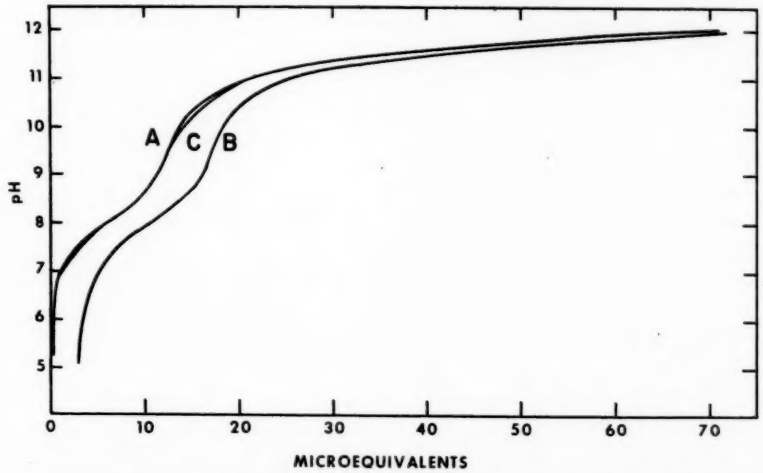

FIG. 3. Titration of clam poison dihydrochloride: (A) initial titration with 0.427 *N* barium hydroxide; (B) back-titration of (A) with 0.479 *N* sulphuric acid following aging at high pH; (C) second titration of (B) with 0.427 *N* barium hydroxide after evaporation from acid.

Preliminary studies on more drastic oxidation of the poisons with periodic acid or potassium permanganate have resulted in the isolation of guanidopropionic acid, urea, carbon dioxide, and ammonia, along with some unidentified products. No success was attained in isolating degradation products, from either the mild or strong oxidations, which represented a sufficiently large portion of the molecule to be of value in determining the chemical structure. Two moles of periodate were consumed very rapidly when the reaction was carried out at pH values between 5 and 13; a third mole was consumed within 24 hours and a fourth after 23 days. Each micromole of poison reduced

17 equivalents of permanganate in 5 minutes at 75° C. Hydrolysis of the dihydro poisons in 12 *N* hydrochloric acid yielded guanidine. Sakaguchi-positive substances were found among the products of oxidation and hydrolysis. The Benedict–Behre and Jaffe tests became negative.

In summary, it is apparent that both reduction with hydrogen or oxidation under aerobic conditions in alkaline solution resulted in the loss of an unsaturated bond essential for toxicity. In the first case it has been demonstrated by countercurrent studies (2) and by studies reported herein that a dihydro derivative of the poison was formed upon reduction. Secondly, no oxygen uptake was apparent for the dihydro derivative, under the conditions described for the mild oxidation of the poison. In addition, the Benedict–Behre and Jaffe tests, which are positive for the poison and negative for the dihydro derivative, were found to be negative for the oxidized poison. Both reduction and oxidation destroyed toxicity. Theoretically only $\frac{1}{2}$ mole of oxygen would be required to bridge a double bond. Because the complete destruction of toxicity required the uptake of 1 mole of oxygen per mole of poison and because the reduced poison took up no oxygen, it is assumed that the oxidation is initiated at the unsaturated bond and that other reactions concurrent with the loss of this bond are taking place. The fact that changes occurred in the ultraviolet absorption and in the optical rotation upon mild oxidation of the poison supports this assumption.

Because reduction of the poison did not change the optical rotation, it appears likely that this unsaturated bond, which is essential to the toxic structure, is not involved in the structure at the optical center.

The strongly basic group would indicate the possibility of an amine or a quaternary ammonium base. However, studies on the alkylation of the poisons gave no evidence for such structures. The absence of an acidic group precludes the existence of a betaine-type base.

The ultraviolet and infrared spectra indicate no aromatic structures, conjugate unsaturation, or isolated carbonyl groups but the elemental ratios require that ring structures be present. Analysis of mussel poison for alkoxyl, alkimide, and C-methyl groups showed the groups to be absent. The Kjeldahl procedure converts all nitrogen to ammonia, which indicates no nitrogen-to-nitrogen bonds. Some of the nitrogen atoms, therefore, must be in heterocyclic structures involving a guanidine group in the cycle to account for the strong basicity of the poison in conjunction with the negative Sakaguchi test. The fact that Sakaguchi-positive compounds are formed upon oxidation or hydrolysis of the poison supports the above indications.

EXPERIMENTAL

Catalytic Hydrogenation of Clam and Mussel Poisons

The catalytic hydrogenation of clam and mussel poisons was carried out in a Warburg respirometer by a procedure similar to that described by Umbreit (5). Platinum black, prepared from platinum dioxide, and platinum black absorbed on charcoal were both effective in catalyzing this reduction in neutral, acid, or alkaline aqueous solutions.

In a typical experiment, platinum black was suspended in 2.0 ml of 0.1 *M* hydrochloric acid in the annulus of a Warburg flask, and 0.40 ml of clam poison dihydrochloride solution (2.50 mg/ml) was placed in the side arm. After the apparatus was gassed with purified hydrogen, the flask was equilibrated at 30°, and the poison solution was tipped into the catalyst. After 440 minutes, the uptake of hydrogen had ceased at 2.65 μmoles, equivalent to 0.95 moles of hydrogen per mole of poison. Four samples of clam poison

had an average uptake of 1.03 moles of hydrogen per mole of poison (range, 0.95–1.16), and eleven samples of mussel poison gave an average of 1.02 moles (range, 0.88–1.25).

An aliquot of a solution of the reduction products from clam poison dihydrochloride (240 μg) was tested for ammonia with the Nessler reagent. A negative reaction was obtained, indicating less than 5 μg of ammonia nitrogen. Countercurrent distribution studies on the reduced poison showed that only one component was produced during the reduction (2). The diffusion coefficient determined in the Northrup diffusion cell was 4.6×10^{-6} cm^2 sec^{-1}, which is similar to that of the poison and therefore indicates a similar molecular weight. The neutralization equivalent was 188, and two titratable groups indicate a molecular weight of 376. Anal. Calc. for $C_{10}H_{21}N_7O_4Cl_2$: C, 32.06; H, 5.66; N, 26.2; O, 17.1; Cl, 18.95%. Found: C, 32.3; H, 5.4; N, 26.3; O (direct), 16.97%.

Infrared Absorption Spectra[6]

The infrared absorption spectra were determined for films, dried from aqueous solutions on polished plates of silver chloride, of the test substance. In order to obtain spectra with a minimum amount of material, the plates were masked with Parafilm (Menasha Products Co., Menasha, Wis.) to reduce the area over which the solution was deposited. Spectra were observed with a Perkin–Elmer Model 12 C spectrophotometer and the results are illustrated in Fig. 1. Comparable spectra were obtained for films of the poison on KRS-5 (potassium thallium bromide), for films deposited from ethyl alcohol solution on sodium chloride,[7] and for pellets with potassium bromide.[8]

Ultraviolet Absorption

All measurements of the ultraviolet spectrum were made in aqueous acid solution (pH 3 to 5) of the poisons in a Model DU Beckman spectrophotometer or a Cary Model 14 spectrophotometer. In special cases when the absorption spectra were measured during the process of alkaline oxidation, small samples were withdrawn from the alkaline reaction flask and measured at the pH of the reaction mixture. The spectra of the poison under various conditions are shown in Fig. 2.

Optical Rotation

Measurements of the optical rotation were made with a Rudolph and Sons No. 80 polarimeter and a sodium-vapor lamp. Normally the measurements were made on aqueous acid (pH 3 to 5) solutions of the poisons in 2-dm tubes. In cases where the rotation was measured during the process of alkaline oxidation, samples were withdrawn from the alkaline reaction flask for each measurement. Where measurements were made under anaerobic conditions the poison solution was kept in the tightly sealed polarimeter tube during the entire period of observation.

Alkaline Oxidation of the Clam Poison

A mixture of 0.5 ml of clam poison solution (4.31 mg/ml; 0.0132 *M*; pH 1.5) and 9.5 ml of 1 *N* sodium hydroxide was allowed to stand in a stoppered flask (no attempt was made initially to exclude oxygen) at room temperature. The solution slowly took on a yellow color, and the formation of the alkaline oxidation product was conveniently

[6] *The spectra were observed by Mr. Edward Weneck except where noted.*

[7] *These spectra were determined by Dr. Robert C. Gore, Stamford Research Laboratories, American Cyanamid Co.*

[8] *The spectra were observed with a Beckman IR2T recording spectrophotometer. U. Schiedt and H. Reinwein. Z. Naturforsch. Part b,* **7b**, *270 (1952); M. M. Stimson and M. J. O'Donnell. J. Am. Chem. Soc.* **74**, *1805 (1952); Perkin–Elmer Instrumental News,* **4**, *No. 3 (1953).*

followed by periodically determining the intensity of ultraviolet absorption at 333 mμ. Maximum absorption (ϵ = 5210) was observed after 4 days. In 0.5 *N* or 0.25 *N* solutions of either sodium or barium hydroxide, 6 days were required for complete reaction, while in 7.5 *N* ammonium hydroxide, the molecular extinction at 333 mμ was only 1500 (30% of maximum) after the same period of time.

When carbon-dioxide-free air was bubbled through 4.0 ml of barium hydroxide solution containing 3.78 mg of clam poison the maximum extinction (ϵ = 5090) was reached in only 9 hours.

The quantitative oxygen uptake involved in the alkaline oxidation reaction was determined by use of the Warburg apparatus. In the bottom of each Warburg flask was placed 0.8 ml of poison solution (4.85 mg/ml), and the side arm contained 0.8 ml of 0.5 *N* barium hydroxide. The center cup contained 0.2 ml of 0.2 *N* sulphuric acid to absorb any ammonia evolved during the reaction. After the flasks had been attached to the individual manometers, they were placed in a water bath maintained at 21°. When the flasks were fully equilibrated, the reaction was started by spilling the contents of the side arm into the bottom of each flask. As the flask was shaken the change in pressure was recorded periodically until the manometric readings remained constant. The results of 12 such determinations showed an average of 0.96 mole of oxygen consumed per mole of poison.

Potentiometric Titrations

Titrations of the poisons and their dihydro derivatives were performed with distilled-water solutions in a 10-ml beaker fitted with a cover provided with holes for the insertion of the glass electrode (No. 290) and calomel electrode (No. 270) of a Beckman pH meter (Model G), a microburette (Gilmont Combination Micro Pipet-Buret, Emil Greiner Co., New York, N.Y.), and a gas delivery tube. Dissolved carbon dioxide was removed from the titration solutions by thoroughly flushing with nitrogen prior to the insertion of the burette. The nitrogen was freed from acidic or basic contaminants by scrubbing with dilute alkali and acid. During the titration, the solution was magnetically stirred and maintained under a stream of the purified nitrogen. Increments of 0.4–0.5 *N* acid or base were added to produce changes in pH of about 0.1–0.2 units. No electrode corrections were applied to the readings taken at high pH because sodium and potassium ions were absent. The temperature of the room varied from 24–25° during the titrations, and no other temperature control was attempted. Values of pK_a were estimated from the data obtained after applying corrections for a distilled water blank.

Titration of Clam Poison Dihydrochloride

A 3.36-ml sample of an aqueous solution containing 4.79 mg of clam poison dihydrochloride was measured into the titration cell. Titration of the sample to pH 12.02 was completed in 24 minutes and required 0.170 ml of 0.427 *N* barium hydroxide. The burette was removed, and the solution was allowed to stand under nitrogen, without stirring, for 2 hours. There was no evidence of a precipitate, indicating that the exclusion of carbon dioxide was complete. A second burette was inserted and the solution was back-titrated with 0.479 *N* sulphuric acid. The final pH of 5.05 was attained in 26 minutes and required 0.145 ml of the reagent. After filtration and lyophilization, the product was dissolved in 5 ml of 0.01 *N* hydrochloric acid and slowly evaporated to dryness (24–48 hours) at room temperature in a vacuum desiccator over potassium hydroxide and phosphoric anhydride. This residue was redissolved in 3.36 ml of water and titrated with 0.168 ml of the standard barium hydroxide to pH 12.02 as described

above. The titration curves are shown in Fig. 3. After again being aged for 2½ hours, the solution was back-titrated with 0.147 ml of the standard acid to pH 4.3. Bioassay of the final solution indicated that 74% of the original toxicity was still present.

Titration of Mussel Poison Dihydrochloride

A 3.27-ml sample of an aqueous solution containing 5.04 mg of mussel poison dihydrochloride was weighed into the titration cell and titrated in the same manner as above. The results were similar to those obtained for clam poison and are illustrated in Fig. 3.

Titration of Dihydro Mussel Poison Dihydrochloride

A sample of dihydro mussel poison dihydrochloride (5.1 mg) was dissolved in 2.0 ml of water and titrated as described above with 0.200 ml of 0.427 *N* barium hydroxide to pH 12.01. The results were similar to those for clam and mussel poisons.

Nonaqueous Titrations

These titrations were carried out with 5-mg samples of clam and mussel poisons according to the procedure given by Seaman and Allen (6) and by Pifer and Wollish (7).

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THE MOLECULAR STRUCTURE OF (−)-N-METHYL-GELSEMICINE HYDRIODIDE, $C_{21}H_{29}O_4N_2I$*

MARIA PRZYBYLSKA AND LÉO MARION

Gelsemicine ($C_{20}H_{26}O_4N_2$), one of the minor alkaloids of *Gelsemium sempervirens* Ait., was discovered by Chou in 1931 (1). It is a monoacidic base which forms a non-basic monobenzoyl derivative so that its basic nitrogen is secondary, a conclusion confirmed by treatment of the base with methyl iodide which gives rise to N-methyl-gelsemicine hydriodide. On hydrogenation over Adams' catalyst, the alkaloid absorbs 3 moles of hydrogen (2) and a comparison of its infrared absorption spectrum with that of its reduction product shows that it must contain a benzene ring since the absorption bands characteristic of that group are no longer present in the spectrum of the reduction product (3). Furthermore, gelsemicine contains two methoxyl groups, and its infrared absorption spectrum contains an absorption band indicative of a carbonyl group (3). It carries a substituent on one of the nitrogens which has been assumed previously to be a methyl group. This sums up the chemical knowledge concerning the alkaloid.

There are two monoclinic forms of N-methyl-gelsemicine hydriodide, one of space group $P2_1$ with two molecules per asymmetric unit and another which crystallizes in space group $C2$ with one molecule per asymmetric unit. The latter compound was chosen for a detailed X-ray analysis. Its unit-cell dimensions are: $a = 16.75$, $b = 9.52$, $c = 14.06$ Å; $\beta = 90°13'$; and $z = 4$.

A total of 2800 reflections were measured and they amounted to 67% of those theoretically available with Cu radiation. The x and z co-ordinates of the iodine atom were derived from two-dimensional Patterson syntheses. After an assumption that the iodine atom lies at $y = \frac{1}{2}$ was made, a three-dimensional Fourier synthesis was evaluated using the phases based only on the iodine atom contributions. The electron-density maps had the symmetry of space group $C2/m$ and the real peaks of the structure were accompanied by mirror-image peaks related to them by planes of symmetry at $y = 0$ and $\frac{1}{2}$. In spite of this difficulty it was possible to locate 22 light atoms of the molecule. Several cycles of refinement using three-dimensional data led gradually to complete solution of the structure. Making use of the anomalous dispersion effects of Cu Kα radiation caused by the iodine atom, it was possible to determine the absolute configuration of this laevorotatory isomer. It is represented by the schematic drawing and the photograph of the molecular model (Fig. 1). The two nitrogen atoms were identified indirectly: that the methyl group is attached to one of them was taken into account, and the position of the second one was considered as established as soon as the oxindole nucleus was recognized (3).

The oxygen atoms were indicated by ρ_o and $(\rho_o - \rho_c)$ maps. Their location was further substantiated by the values of bond lengths, which are in good agreement with the nominal bond lengths.

The reliability factor, R, for all three-dimensional data is 0.16, whereas for the two zones ($h0l$) and ($0kl$), it is 0.16 and 0.10 respectively. The R factor for the centrosymmetric ($h0l$) zone is expected to be higher than for the non-centrosymmetric ($0kl$) data (4).

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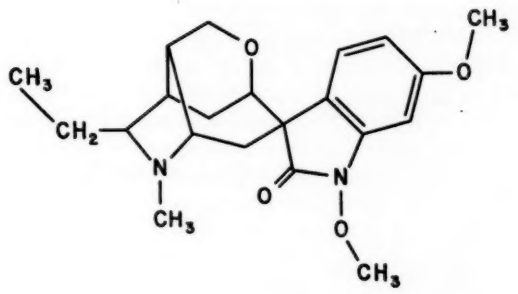


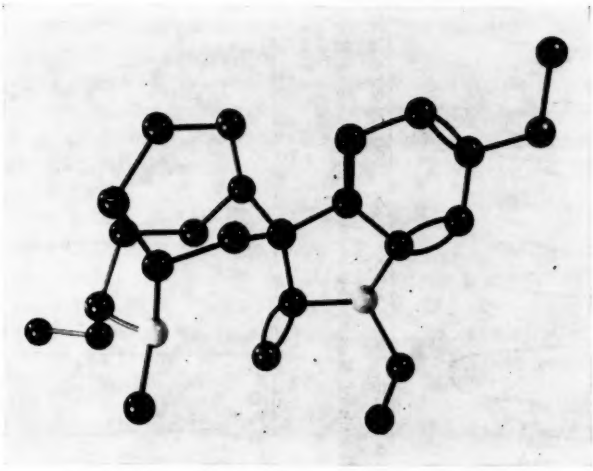
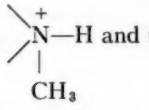


FIG. 1. Molecular model of (−)-N-methyl-gelsemicine.

A full account of this work will be published at a later date.

On comparison of the skeleton of N-methyl-gelsemicine and gelsemine (5) we find that they are very similar, but the skeleton of N-methyl-gelsemicine has one carbon atom less which leads to a somewhat simplified structure.

One unusual feature that deserves comment is the presence of a methoxyl group linked to the oxindole nitrogen. It has been shown chemically that the molecule of gelsemicine contains two methoxyl groups. The base forms salts which are normal and not of the anhydronium type as shown by their infrared spectra and by the recovery from these salts of the intact base still containing two methoxyl groups. Hence, the two-atom group attached to the carbon next to the basic nitrogen cannot be a methoxyl group.

The distance between the nitrogen ion  and the oxygen of the carbonyl group

in N-methyl-gelsemicine hydriodide is 2.77 Å. Since the hydrogen atom attached to the nitrogen is directed towards the oxygen atom, an intramolecular hydrogen-bond formation is indicated. Additional evidence is supplied by the infrared absorption spectrum, Fig. 2,

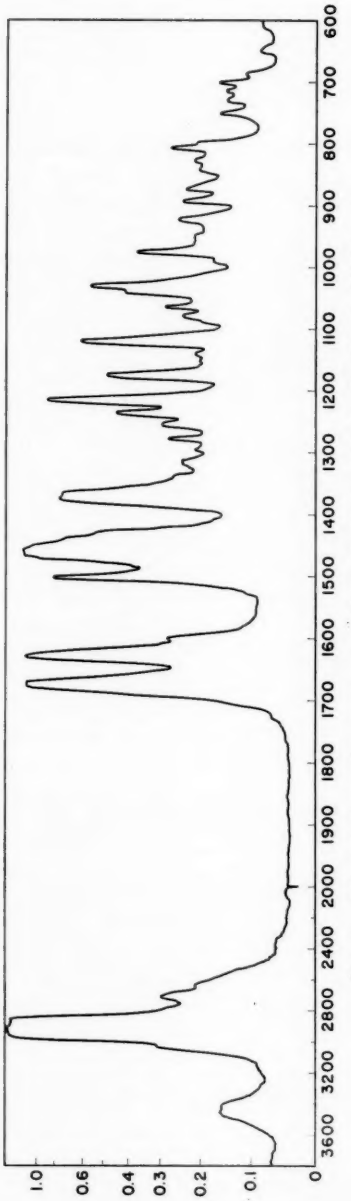


FIG. 2. Infrared absorption spectrum of N-methyl-gelsemicine hydriodide in Nujol mull.

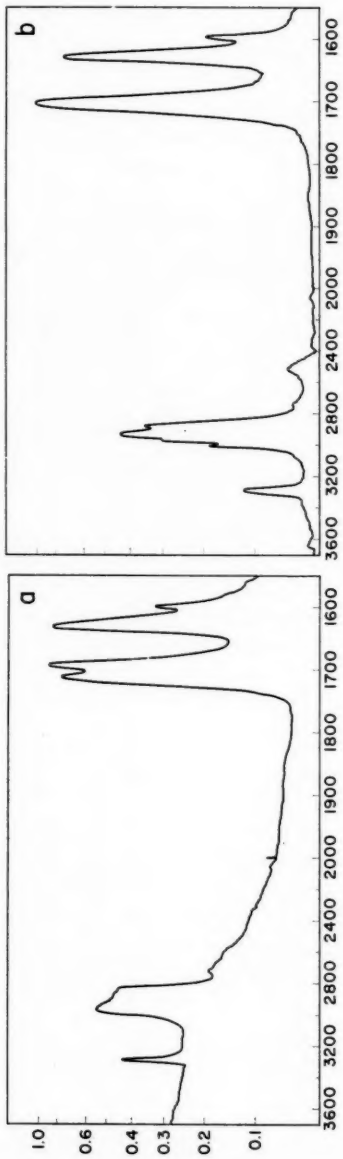


FIG. 3. Infrared spectra of gelsemicine: (*a*) in Nujol mull; (*b*) in chloroform solution.

which shows a markedly broadened band at ~3340 cm^{-1} and a considerable shift in the absorption of the carbonyl band, with a maximum at 1674 cm^{-1}.

It is of interest that the infrared spectrum of gelsemicine itself in Nujol mull shows a double peak in the carbonyl region, whereas a single maximum is obtained in chloroform solution (Fig. 3). It seems that the C=O group, only in some, but not all molecules of the crystalline gelsemicine, is involved in the hydrogen-bond formation. The C=O bond in the spectrum in chloroform solution lies between the position of the two peaks of the Nujol mull spectrum and this indicates that the C=O groups of all the molecules may be involved in H bonding with the C—H group of chloroform. Although crystalline gelsemicine itself has not been X-ray analyzed, some support to this explanation is given by the fact that in gelsemicine hydrobromide hemihydrate, N-methyl-gelsemicine hydrobromide tetrahydrate (6), and also in N-methyl-gelsemicine hydriodide of space group $P2_1$, the asymmetric unit was found to consist of two molecules which are, therefore, crystallographically not equivalent. This tendency of the compound to crystallize in pairs of molecules may well be due to the shape of its skeleton which contains a large planar oxindole group. In this connection, it is noteworthy that the infrared spectrum of dihydrogelsemine in chloroform also shows a single peak in the carbonyl region, although in a mull, a double peak is obtained.

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THE CLEAVAGE OF ETHERS BY *t*-BUTYLMAGNESIUM BROMIDE AND COBALTOUS CHLORIDE[1]

E. Eisenbraun[2] and C. B. Purves

In connection with attempts to break ether bonds in wood lignins by mild methods, the effect of the above Grignard – cobaltous chloride reagent on 2-methoxydiphenyl ether was briefly studied. Kharasch and Huang (1) discovered that the addition of anhydrous cobaltous chloride enabled a Grignard reagent to cleave many diaryl and benzyl aryl ethers to a mixture of the corresponding hydrocarbon and phenol in 3 to 4

[1]*Financed in part from a National Research Council of Canada grant.*
[2]*Holder of a Union Carbide Corporation (Visking Division) Fellowship in 1958–59. Present address: Arnold Hoffman and Company, Incorporated, Providence, Rhode Island, U.S.A.*

hours at 36°, instead of in 15 to 18 hours at 180–200°. No scission of methyl phenyl and ethyl phenyl ethers was observed. *n*-Butylmagnesium bromide was effective (1, 2), but Tomita and Watanabe (3) found that the yield of cleavage products was somewhat greater when the tertiary isomer was used; the replacement of diethyl ether by tetrahydrofuran as the solvent increased the yield threefold.

On the present occasion, eight experiments with 2-methoxydiphenyl ether were carried out, the Grignard reagent being made from either *n*-butyl or *t*-butyl bromide, and the solvent being ether or tetrahydrofuran. The best result was obtained with *t*-butylmagnesium bromide in tetrahydrofuran. With a 30-fold molar excess, 36% of the original substance was recovered unchanged together with 35% of phenols, corresponding to a degree of cleavage of about 60%. This yield corresponded closely to the value of 62% obtained by Tomita and Watanabe (3) for the same compound. Both guaiacol and phenol were isolated as the pure, crystalline benzoates, and cleavage therefore occurred on both sides of the diphenyl ether oxygen atom, in agreement with the work of Kharasch and Huang (1). Tomita and Watanabe (3) thought that this ether oxygen atom was completely retained by the guaiacol (benzene presumably was the only other product), but an attempt to repeat their isolation of the guaiacol as the *p*-nitrobenzoate gave a crystalline product which probably contained some of the phenol derivative. Fruitless attempts were also made to detect catechol, by paper chromatography, among the products, and this negative result agreed with the claim that methyl phenyl ether groups were not affected by the reagent.

t-Butylmagnesium bromide – cobaltous chloride, when warmed with a suspension of fully methylated spruce formaldehyde periodate lignin (4) in tetrahydrofuran, produced 2% of phenolic material which resinified too rapidly to be examined in detail, together with a little neutral, high-boiling oil. There was no clear evidence that any cleavage of ether groups occurred in this lignin. When the experiment was repeated with fully methylated spruce periodate lignin, the resulting 4% of ether-soluble material probably contained guaiacol, and the 90% of residual lignin increased in methoxyl content from 17.2 to 18.5% on remethylation with diazomethane. In this case, about 0.7% of phenolic groups might have been liberated by cleavage.

EXPERIMENTAL

Materials

Tetrahydrofuran, boiling correctly at 76°, was used not more than 2 days after purification by distillation in succession from solid sodium hydroxide, metallic sodium, and lithium aluminum hydride. The Grignard reagent was made from pure *t*-butyl bromide and dry, polished magnesium ribbon (5, 6); the use of powdered crystals of sublimed magnesium, obtained through the courtesy of Dow Chemical Company of Canada, yielded a particularly clear, colorless reagent (7). Dilution of an aliquot with a known volume of standard acid, and a back titration with standard alkali to a phenolphthalein end point (8), established the molarity of the reagent. The yield amounted to 50% of the magnesium when tetrahydrofuran was the solvent, whereas the reported yield was 33% (6).

A published method (9) of preparing anhydrous cobaltous chloride was modified by heating the pure hexahydrate for 2 days at 45° and 20 mm pressure, and then gradually raising the temperature to 220° (1 mm). The light pink of the partly dehydrated product changed through dark blue to the pale blue color of the anhydrous salt, which was

extremely finely divided. Ullmann and Stein's method (10) was used to synthesize 2-methoxydiphenyl ether, which was recrystallized from hexane and redistilled under diminished pressure; m.p. 76°, the recorded value being 78°.

Cleavage of 2-Methoxydiphenyl Ether

The apparatus consisted of a three-necked flask equipped with ground-glass joints, a magnetic stirrer, a reflux condenser, leads for dry nitrogen gas, and an L-shaped tube, revolving in a ground-glass joint, for the addition of the anhydrous cobaltous chloride. This tube could be warmed externally to prevent the condensation of solvent, which made the finely divided chloride sticky. A solution of 0.098 mole of *t*-butylmagnesium bromide in tetrahydrofuran was filtered through dry glass wool into the flask, the volume plus rinsings being 80 ml. After 1.3 g (6.5 mmoles) of 2-methoxydiphenyl ether had been added, the apparatus was flushed with nitrogen, and solution was completed by gentle heating and stirring. The cobaltous chloride, 4.68 g (36 mmoles), was then added from the L tube in 12 successive portions at 15-minute intervals, after which the mixture was heated for 3 hours under reflux.

The mixture was acidified near 0° with 4 *N* hydrochloric acid and thoroughly extracted with ether, and the extract was re-extracted with 5% aqueous sodium hydroxide (1). A neutral fraction, 0.50 g, isolated from the ether, yielded 0.375 g (29%) of twice-recrystallized 2-methoxydiphenyl ether with the correct melting point and mixed melting point. Benzoylation (5) of the product extracted by the alkali, 0.50 g, yielded 0.72 g of crystals, m.p. 51–56°, which on recrystallization from ethanol produced 0.45 g of guaiacol benzoate, m.p. 57–58°, and mixed m.p. 56–58°. Evaporation of the ethanol mother liquors left a gum which on standing deposited 0.07 g (0.35 mmole) or 5% of crystals melting at 63–66°, increased to m.p. 69° by recrystallization. Authentic phenyl benzoate had this melting point, and a mixed melting point with the crystals was not depressed. A further 0.08 g of pure guaiacol benzoate, isolated from the final mother liquors, brought the total yield of this product to 2.3 mmoles or to 35% of theory. Since a control benzoylation of pure guaiacol gave only a 50% yield of the pure, recrystallized benzoate, the yield of guaiacol actually produced in the cleavage was probably near 70%.

In another experiment, a small portion of the alkali-soluble product from the scission of 1.04 g (5.2 mmoles) of 2-methoxydiphenyl ether was chromatographed on paper for 20 hours with butanol – 2% aqueous ammonia as the solvent and a ferric chloride mixture (4) as the spray. Only the spot characteristic of guaiacol, R_f 0.95, was present, and there was no spot in the catechol position, R_f 0.87. The remainder of the product, when esterified with *p*-nitrobenzoyl chloride in pyridine (5), yielded 0.47 g of material which was recrystallized once from ethanol. Although the crystals, 0.26 g, melted rather sharply at 96–97°, many recrystallizations from ethanol and methanol separated them into 0.15 g melting at 86–92°, and 0.03 g melting at 98–100°. Since the melting points of the *p*-nitrobenzoates of guaiacol and phenol were 97° and 102°, respectively, the above product was probably an incompletely resolved mixture of these two compounds.

Attempted Scission of Fully Methylated Lignins

A 1.4-g sample of dry, finely divided spruce formaldehyde periodate lignin, fully methylated to OCH_3, 15.0% (4), was suspended in 90 ml of tetrahydrofuran containing 80 mmoles of *t*-butylmagnesium chloride. Six grams of anhydrous cobaltous chloride was added in 12 portions during 3 hours, and the subsequent heating under reflux was for 3 hours. The cold, acidified suspension was then extracted with ether, and the aqueous

residue was dialyzed against distilled water. The non-dialyzed portion yielded 1.20 g of a precipitate. Found: ash, 0.7%; OCH_3, 12.5%, not altered by methylation with diazomethane. The ether extract contained 0.2 g of a neutral, high-boiling oil, and 0.03 g of alkali-soluble material with R_f 0.21 on paper chromatograms.

The experiment was repeated with 1.42 g of spruce periodate lignin fully methylated to OCH_3, 17.6; ash, 1.2% (4). Dialysis of the aqueous portion of the reaction mixture yielded 1.29 g of insoluble material. Found: ash, 0.2%; OCH_3, 17.2%, increased to 18.6% by remethylation with diazomethane. The neutral ether-soluble oil, 0.085 g, was volatile enough to leave only 0.02 g of residue when heated at 100° *in vacuo*. When the ether-soluble phenols, 0.06 g, were chromatographed on paper only the spot of R_f 0.95 ertaining to guaiacol was observed.

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THE DECOMPOSITION OF HYDROGEN PEROXIDE IN THE PRESENCE OF HYDROGEN

R. R. BALDWIN, D. BOOTH,* AND D. BRATTAN†

In a paper (1) presented at the Eighth International Combustion Symposium at Pasadena in September 1960, we reported a marked acceleration of the decomposition of H_2O_2 in the presence of H_2. The studies were carried out using a flow system at atmospheric pressure, the partial pressure of H_2O_2 varying from 0.2–1.0 mm Hg, and the partial pressure of H_2 varying from 10–760 mm Hg. In such a system, where the H_2/H_2O_2 ratios were relatively high, the experimental results are interpreted precisely by the following simple scheme:‡

$$H_2O_2 + M' = 2OH + M' \qquad [7]$$

$$OH + H_2 = H_2O + H \qquad [1]$$

$$H + H_2O_2 = H_2O + OH \qquad [14]$$

$$H + H_2O_2 = H_2 + HO_2 \qquad [14a]$$

$$OH + H_2O_2 = H_2O + HO_2 \qquad [15]$$

$$2HO_2 = H_2O_2 + O_2. \qquad [10]$$

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‡The numbering scheme is that used in earlier papers.

Reactions [15] and [14a] represent the termination reactions dominant at low and high H_2/H_2O_2 ratios respectively, and from the results, values for k_{15}/k_1 of 6.0±0.5 and for k_{14a}/k_{14} of approximately 0.1 are obtained. In the presence of O_2, the termination reaction [4] also becomes important.

$$H + O_2 + M = HO_2 + M \qquad [4]$$

The results and discussion given by Forst (2) in a recent paper in this journal seem, at first sight, to be at variance with the simple mechanism given above, and we wish to show that Forst's results are, in fact, consistent with the above mechanism. Forst's results, all obtained at 431.5° C, fall into three sections, which are given below as points (a), (b), and (c).

(a) His Fig. 1 gives the pressure–time curve for the decomposition of H_2O_2 alone (9.6 mm Hg pressure) and in the presence of 19.2 and 96.2 mm Hg of H_2. With pure H_2O_2, the limiting pressure rise of 4.5 mm is close to the theoretical value (4.8 mm Hg), but the pressure rise decreases to 2.6 and 1.5 mm Hg respectively in the presence of H_2.

(b) Figure 2 gives the initial rate (mm Hg of O_2 per sec) of decomposition of 9.6 mm of H_2O_2 in the presence of 10–77.5 mm Hg of H_2.

(c) Figure 3 gives the initial rate of decomposition as a function of H_2O_2 concentration over the range 3–16 mm Hg, the pressure of H_2 being 57.9 mm Hg.

To allow for the different coefficients of H_2 and H_2O_2 in reaction [7], the rate of [7] may be written

$$R = k_{7P}[P]^2 + k_{7H_2}[H_2][P], \qquad [i]$$

where [P] is the concentration of H_2O_2. Forst* evaluated the ratio k_{7H_2}/k_{7P} as 0.18 by assuming that the ratio k_{7H_2}/k_{7He} is the ratio of the calculated collision frequencies (1.64), the ratio k_{7He}/k_{7P} having been determined as 0.11 (3). Use of this value for k_{7H_2}/k_{7P} gives the linear increase in rate with added H_2, as found experimentally, but the calculated rate increases slower than the experimental rate as H_2 is added. The same is found with the data of Fig. 3, and Forst concludes that some chemical effect of H_2 is also present. Since reactions [1], [14], and [14a] only increase the rate of H_2O_2 decomposition and do not affect the rate of O_2 formation, he concludes that other reactions must occur.

Studies of the H_2–O_2 reaction, however, indicate that the ratio k_{4H_2}/k_{4He} is almost twice the calculated collision frequency ratio and that the ratio of the values of k_4 is only equal to the collision frequency ratio if both gases are monatomic or if both gases are diatomic (4). A more likely estimate of the ratio of k_{7H_2}/k_{7P}, therefore, is obtained by assuming that the relative coefficients of H_2 and N_2 are the same in reactions [4] and [7]. Since $k_{4N_2}/k_{4H_2} = 0.43$ (4), and $k_{7P}/k_{7N_2} = 6$–7 (1, 5), k_{7H_2}/k_{7P} should be approximately 0.33–0.38.

A value in agreement with this estimate can be obtained from Forst's results if they are interpreted by the Baldwin–Brattan mechanism. This mechanism, which is also consistent with independent studies of the hydrogen–oxygen reaction (6, 7), gives the rate expressions

$$-\frac{d[P]}{dt} = \frac{2k_7[P][M']\{k_1[H_2]+k_{15}[P]\}}{\alpha k_1[H_2]+k_{15}[P]}, \qquad [ii]$$

$$\frac{d[O_2]}{dt} = k_7[P][M'], \qquad [iii]$$

The authors are grateful to Dr. Forst for explaining the basis of this calculation.

where $\alpha = k_{14a}/(k_{14}+k_{14a})$. Since Forst followed the decomposition by measurement of pressure change, his rate measurements are represented by [iii], which can be written in a similar manner to [i] and predicts the linear increase in rate with H_2 concentration shown in Fig. 2 of Forst's paper. From the gradient and intercept of this line, experimental values of k_{7H_2} and k_{7P} can be obtained, the ratio being 0.30. These experimental values can now be used to predict the variation of initial rate with H_2O_2 concentration at constant H_2 concentration. Figure 1 shows the experimental points taken from Forst's

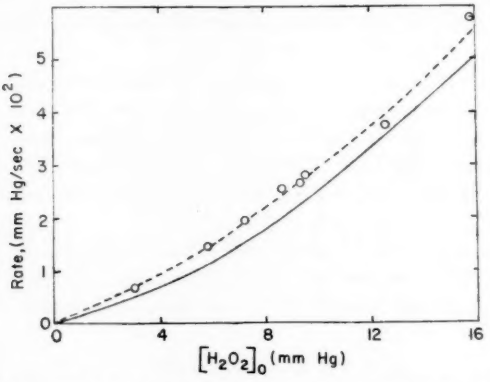

FIG. 1. Decomposition of H_2O_2 in the presence of hydrogen at 431.5° C (initial concentration H_2 = 57.9 mm Hg): O experimental points (Forst (2), Fig. 3); —— calculated curve (Forst (2), Fig. 3); - - - calculated curve (Baldwin–Brattan mechanism).

paper (Fig. 3), the calculated curve using Forst's ratio of 0.18, and the corresponding curve using a ratio of 0.30. This latter ratio thus accounts for both sets of experimental results obtained by Forst, and is in sufficiently close agreement with the estimate of 0.33–0.38 above, particularly since allowance for surface decomposition, admitted by Forst to be significant at this temperature, would increase the value of k_{7H_2}/k_{7P}.

From [ii] and [iii],

$$\frac{-d[P]}{d[O_2]} = \frac{2(k_1[H_2]+k_{15}[P])}{\alpha k_1[H_2]+k_{15}[P]}.$$

Integration* between the initial peroxide concentration $[P_0]$ and zero gives the total amount of O_2 formed as

$$2[O_2]_f = [P_0]-(1-\alpha)R[H_2]\log_e\frac{[P_0]+R[H_2]}{R[H_2]},$$

where $R = k_1/k_{15} = 1/6$ (1). Insertion of the values used in Fig. 1 of Forst's paper, $[H_2O_2]$ = 9.6 mm Hg, $[H_2]$ = 19.2 and 96.2 mm Hg, gives $[O_2]_f$ as 2.8 and 1.4 mm Hg, in close agreement with the experimental values of 2.6 and 1.5 mm Hg respectively.

It may thus be concluded that the Baldwin–Brattan mechanism gives a completely satisfactory account of Forst's observations without the necessity of postulating further reactions.

**This integration assumes that reaction [4] may be neglected and that $[H_2]$ is constant. From the known (1) values of k_2/k_4 and k_{14}/k_2, it can be shown that, under Forst's experimental conditions, reaction [4] will not compete with [14] until $[H_2O_2]$ = 0.1 mm Hg. The error resulting from the assumption that $[H_2]$ is constant can also be neglected.*

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CRYSTALLOGRAPHIC DATA FOR ETHYL 3,5-DINITROBENZOATE

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An analysis of the crystal structure of ethyl 3,5-dinitrobenzoate was undertaken to investigate any deviations of the nitro groups from coplanarity with the aromatic ring. However, the structure has proved too complex for a complete determination of the molecular parameters, and since the crystallographic data might be useful, they are outlined in this note.

Crystals of ethyl 3,5-dinitrobenzoate, prepared by reaction of 3,5-dinitrobenzoyl chloride with ethanol, and recrystallized from ethanol, are pale yellow needles elongated along the *b*-axis, with the (001) plane developed and a smaller (100) face. The density was measured by flotation in aqueous potassium iodide solution, and the unit-cell dimensions and space group were determined from rotation and oscillation photographs of a crystal rotating about the *b*-axis, *h*0*l* and *h*1*l* Weissenberg films, and *hk*0 and 0*kl* precession films.

Crystal Data

Ethyl 3,5-dinitrobenzoate: $C_9H_8N_2O_6$; molecular weight = 240.2; melting point = 92–94° C.

Monoclinic; $a = 18.36 \pm 0.05$, $b = 4.78 \pm 0.01$, $c = 13.82 \pm 0.03$ Å; $\beta = 119.7° \pm 0.5°$.

Volume of the unit cell = 1054 Å^3.

Density: calculated (with $Z = 4$) = 1.505, measured = 1.511 g cm^{-3}.

Absorption coefficient for X rays, $\lambda = 1.542$ Å, $\mu = 12.9$ cm^{-1}.

Total number of electrons per unit cell = $F(000) = 496$.

Examination of the films revealed that *h*0*l* reflections are systematically absent when *h* is odd. Of the 0*k*0 reflections only 040 was observed, so that a screw axis parallel to *b* was indicated. Since only one 0*k*0 reflection was observable the presence of the 2_1 axis was not definitely established, but the $N(z)$ intensity distribution (1) for the *h*0*l* zone corresponded to a centrosymmetric projection. These facts, taken together, suggested that the space group was probably $P2_1/a$.

The intensities of the *h*0*l* reflections were measured by the Weissenberg multiple-film technique (2), with Cu $K\alpha$ radiation, and the values of the structure amplitudes were

derived by the usual formulae. One hundred and three reflections were recorded. In space group $P2_1/a$ there is one molecule in the asymmetric crystal unit, and the orientation of this molecule in the unit cell was deduced by Fourier-transform methods and from the intramolecular peaks in the Patterson projection. However, it was impossible to pack molecules in this orientation into the unit cell in space group $P2_1/a$, so that it seemed that there might be two molecules in the asymmetric unit arranged in space group Pa, the 010 and 030 reflections being by chance too weak to be observed, and the $N(z)$ test being misleading (a not uncommon occurrence). Alternatively there might be some type of disordered arrangement of molecules. The problem, therefore, seemed too complex for solution by the usual trial and error methods, and no further detailed structural analysis is proposed. The structure-factor data have been recorded (3).

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A NEW METHOD FOR THE DEOXYGENATION OF AROMATIC *N*-OXIDES

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In the course of a study of the cyclization of some nitrophenylpyridine derivatives with ferrous oxalate it was observed that when 2-*o*-nitrophenylpyridine-*N*-oxide was heated with this reagent the only product isolated was pyrido[1,2-*b*]indazole (1). It was also shown that the first stage in this reaction was probably the elimination of the *N*-oxide grouping; 2-phenylpyridine-*N*-oxide itself was deoxygenated under these conditions but was unaffected in the absence of the ferrous oxalate.

The scope of this deoxygenation has now been extended to a number of other pyridine-*N*-oxide derivatives and to *N*,*N*-dimethylaniline-*N*-oxide. The results are summarized in the table.

N-Oxide	Yield of tertiary amine, %
Pyridine-*N*-oxide	64
2-Picoline-*N*-oxide	62
3-Picoline-*N*-oxide	72
2-Aminopyridine-*N*-oxide	45
2-Phenylpyridine-*N*-oxide	63
N,*N*-Dimethylaniline-*N*-oxide	55 (crude)

This new procedure may well be of value, particularly in cases (e.g. when an amino group is present) where other methods currently in use (such as that involving phosphorus trichloride) may not be employed. Its limitations include applications to (*a*) those substances containing groupings, e.g. nitro, which are attacked by ferrous oxalate; and (*b*)

probably aliphatic tertiary amine *N*-oxides, which are known (see ref. 2) to undergo elimination of the amine group by the action of heat and to give an olefin.

EXPERIMENTAL

The *N*-oxide was mixed with 4 molar equivalents of ferrous oxalate dihydrate and granulated lead (ca. 6 g/g of *N*-oxide), and the mixture ((*a*) in a flask arranged for distillation for *N*-oxides of liquid amines, or (*b*) in a flask fitted with a condenser for *N*-oxides of solid amines) was heated in a metal bath at 300° (bath temperature) for 30 minutes. The product isolated by distillation (in case (*a*)), or by ether extraction of the reaction mixture (in case (*b*)), was dried overnight over potassium hydroxide pellets. The identity of the products was established by comparison of their infrared spectra with those of authentic samples of the bases. The picrates of the products were prepared in the cases of pyridine-, 2-picoline-, 3-picoline-, and 2-phenylpyridine-*N*-oxides, and their identity confirmed by melting point and mixed melting point determinations. 2-Aminopyridine, as isolated from this reaction, was recrystallized from light petroleum (40–60°) and had m.p. 57–59°, which was not depressed on admixture with an authentic sample.

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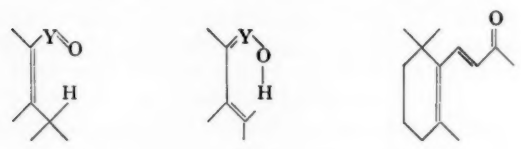
THE IRRADIATION OF β-IONONE

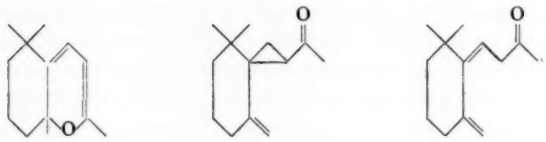
P. de Mayo, J. B. Stothers, and R. W. Yip

Study of the literature suggests that the irradiation of substances containing the system (I) where Y is carbon and (or) a hetero atom(s) leads to (reversible) hydrogen transfer and the formation of (II) which may then be further transformed. In those cases noted there is the possibility of $n \rightarrow \pi^*$ excitation, though this may not be a requirement. The irradiation of α,β-unsaturated ketones ((I), Y = C·CH_3) gives, for example, the β,γ-unsaturated isomer (1). All the requirements of the *o*-nitrobenzaldehyde ((I), Y = NO) – *o*-nitrosobenzoic acid conversion and related changes are met by the postulation of an intermediate ketene formed by hydrogen transfer (2). Recently it has been shown that substituted benzophenones ((I), Y = C·Ph) are enolized by irradiation (3).

β-Ionone (III) on irradiation has been shown (4) to give (reversibly) the pyran (IV) as main product, together with a by-product which was tentatively attributed the structure (V). The alternative (VI) was considered and rejected on reasonable but negative evidence. Since β-ionone falls into the class of substance under present consideration, by vinylogous extension, (VI) seemed mechanistically much more favored. We have repeated the preparation and determined the n.m.r. spectrum (Fig. 1), which was not available at the time of the earlier work.



(I) (II) (III)



(IV) (V) (VI)

At 60 Mc/sec in dilute carbon tetrachloride solution, the following peaks are found (in p.p.m. from internal tetramethylsilane): 1.06 (singlet), $(CH_3)_2C<$; 2.04 (singlet), $CH_3—\underset{|}{C}=O$: 3.16 (doublet, $J = 7.1$ cycles/sec), $=CH—\underline{CH_2}—C=O$; 5.33 (triplet, $J =$

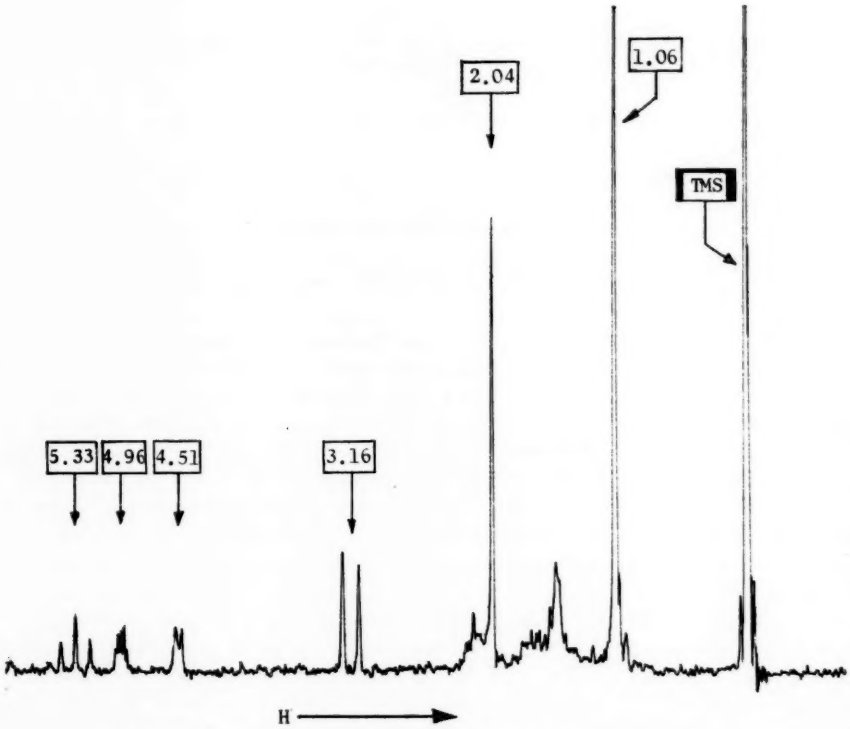


FIG. 1. 60 Mc/sec spectrum of the irradiation product (VI) of β-ionone.

7.1 cycles/sec), $=\underline{CH}-CH_2-C=O$; 4.51, 4.96 (multiplets), $=CH_2$. From the splitting observed in the latter two bands, it was found that $J = 2.6$ cycles/sec for the geminal coupling, while the lower band clearly showed a long-range interaction with $J = 1.3$ cycles/sec. It is to be noted that no absorption characteristic of the cyclopropane methylene group required by (V) is observed. These results conclusively establish the correctness of (VI).

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NOTES TO CONTRIBUTORS

Canadian Journal of Chemistry

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